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A TEXT-BOOK

OF

BACTERIOLOGY

BY

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RIO DE JANEIRO, OF THE SOCIÉTÉ FRANÇAISE D'HYGIÈNE, ETC., ETC.

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Second Revised Edition

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PREFACE.

THE writer's Manual of Bacteriology, published in 1892, has been very favorably received both in this country and abroad, but its usefulness has no doubt been to some extent restricted by the size and expense of the volume. The following is an extract from the preface of the Manual:

"A Manual of Bacteriology, therefore, which fairly represents the present state of knowledge, will consist largely of a statement of facts established by experimental data, and cannot fail to be of value to physicians and to advanced students of bacteriology as a work of reference. The present volume is an attempt to supply such a manual, and at the same time a *text-book of bacteriology for students* and guide for laboratory work. That portion of the book which is printed in large type will, it is hoped, be found to give an accurate and sufficiently extended account of the most important pathogenic bacteria, and of bacteriological technology, to serve as a text-book for medical students and others interested in this department of science. The descriptions of non-pathogenic bacteria, and of the less important or imperfectly described species of pathogenic bacteria, are given in smaller type."

For the benefit of students of medicine and others who do not care especially for the detailed descriptions of non-pathogenic bacteria and the extensive bibliography contained in the Manual, this TEXT-BOOK OF BACTERIOLOGY is now published. It comprises that portion of the Manual above referred to as printed in large type, revised to include all important additions to our knowledge of the pathogenic bacteria since the original date of publication.

1896.

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PREFACE TO SECOND EDITION.

AT the request of the publishers the author has again undertaken a revision of his Manual of Bacteriology, published in 1892. This is practically a third edition of that work, although the title was changed in 1896, and it now appears as a second edition of a TEXT-BOOK OF BACTERIOLOGY.

Considerable additions have been made to the present edition, including a section on "Protective Inoculations in Infectious Diseases," and one on the "Bacteria of Plant Diseases." In order that the size of the work might not be materially increased, descriptions of species imperfectly described, or of minor importance, have been omitted. In the Manual of Bacteriology an attempt was made to include all species or distinct varieties which had been described by competent bacteriologists up to that date, and to give a very full bibliography of the subject. It was found to be impracticable to follow this plan in bringing out a second edition as it would have called for two large volumes instead of one, and the limited demand for such a work would probably have made it a losing venture for the publishers. In the TEXT-BOOK, therefore, the bibliography and the descriptions of many non-pathogenic species were omitted. The Manual is now out of print, and those who have use for a comprehensive work, in which an attempt has been made to include all species described up to date of publication, are referred to Migula's "System der Bakterien" (Gustav Fischer, Jena, 1900).

WASHINGTON, *May 27th, 1901.*

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PART FIRST.

CLASSIFICATION, MORPHOLOGY, AND GENERAL BACTERIOLOGICAL TECHNOLOGY.

I. Historical. II. Classification. III. Morphology. IV. Staining Methods. V. Culture Media. VI. Sterilization of Culture Media. VII. Cultures in Liquid Media. VIII. Cultures in Solid Media. IX. Cultivation of Anaërobic Bacteria. X. Incubating Ovens and Thermo-Regulators. XI. Experiments upon Animals. XII. Photographing Bacteria.

PART FIRST.

I.

HISTORICAL.

It is probable that Leeuwenhoeck, "the father of microscopy," observed some of the larger species of bacteria in fæces, putrid infusions, etc., which he examined with his magnifying glasses (1675), but it was nearly a century later before an attempt was made to define the characters of these minute organisms and to classify them (O. F. Müller, 1773).

In the absence of any reliable methods for obtaining pure cultures, it is not surprising that the earlier botanists, in their efforts to classify microörganisms, fell into serious errors, one of which was to include under the name of infusoria various motile bacteria. These are now generally recognized as vegetable organisms, while the *Infusoria* are unicellular animal organisms.

Ehrenberg (1838), under the general name of *Vibrioniens*, describes four genera of filamentous bacteria, as follows:

1. *Bacterium*—filaments linear and inflexible; three species.
2. *Vibrio*—filaments linear, snake-like, flexible; nine species.
3. *Spirillum*—filaments spiral, inflexible; three species.
4. *Spirochæte*—filaments spiral, flexible; one species.

These vibrioniens were described by Ehrenberg as "filiform animals, distinctly or apparently polygastric, naked, without external organs, with the body uniform and united in chains or in filiform series as a result of incomplete division."

Dujardin (1841) also placed the vibrioniens of Ehrenberg among the infusoria, describing them as "filiform animals, extremely slender, without appreciable organization, and without visible locomotive organs."

Charles Robin (1853) suggested the relationship of Ehrenberg's vibrioniens with the genus *Leptothrix*, which belongs to the algæ; and Davaine (1859) insisted that the vibrioniens are vegetable organisms, nearly allied to the algæ. His classification will be found in the "Dictionnaire Encyclop. des Sciences Médicales," art. "Bactéries" (1868). This view is also sustained by the German botanist Cohn and is now generally accepted.

Spallanzani, in 1776, endeavored to show by experiment that the generally received theory of the spontaneous generation of microörganisms in organic liquids was not true. This he did by boiling putrescible liquids in carefully sealed flasks. The experiment was not always successful, but in a certain number of instances the liquids were sterilized and remained unchanged for an indefinite period. The objection was raised to these experiments that the oxygen of the air was excluded by hermetically sealing the flasks, and it was claimed, in accordance with the views of Gay-Lussac, that free admission of this gas was essential for the development of fermentation.

This objection was met by Franz Schulze (1836), who admitted air to boiled putrescible liquids by drawing it through strong sulphuric acid, in which suspended microörganisms were destroyed. He thus demonstrated that boiled solutions, which, when exposed to the air without any precautions, quickly fell into putrefaction, remained unchanged when freely supplied with air which had been passed through an agent capable of quickly destroying all living organisms contained in it.

Schwann (1839) demonstrated the same fact by another method. Air was freely admitted to his boiled liquids through a tube which was heated to a point which insured the destruction of suspended microörganisms. The same author is entitled to the credit of having first clearly stated the essential relation of the yeast plant—*Saccharomyces cerevisiæ*—to the process of fermentation in saccharine liquids, which results in the formation of alcohol and carbonic acid.

Helmholtz, in 1843, repeated the experiments of Schwann with calcined air, and arrived at similar results—*i.e.*, he found that the free admission of calcined air to boiled organic infusions did not produce fermentation of any kind.

It was objected to these experiments that the air, having been subjected to a high temperature, had perhaps undergone some chemical change which prevented it from inaugurating processes of fermentation.

This objection was met by Schröder and Von Dusch (1854) by a very simple device which has since proved to be of inestimable value in bacteriological researches. These observers showed that a loose plug of cotton, through which free communication with the external air is maintained, excludes all suspended microörganisms, and that air passed through such a filter does not cause the fermentation of boiled organic liquids.

The experiments of Pasteur and of Hoffman, made a few years later, showed that even without a cotton filter, when the neck of the flask containing the boiled liquid is long drawn out and turned downward, the contents may be preserved indefinitely without change. In this case suspended particles do not reach the interior of the flask, as there is no current of air to carry them upward through its long-drawn-out neck, and they are prevented by the force of gravity from ascending.

Tyndall showed at a later date that in a closed chamber, in which the air is not disturbed by currents, all suspended particles settle to the floor of the chamber, leaving the air optically pure, as is proved by passing a beam of light through such a chamber.

Notwithstanding the fact that the experimenters mentioned had succeeded in keeping boiled organic liquids sterile in flasks to which the oxygen of the air had free access, the question of the possibility of spontaneous generation—*heterogenesis*—still remained unsettled, inasmuch as occasionally a development of bacterial organisms did occur in such boiled liquids.

This fact was explained by Pasteur (1860), who showed that the generally received idea that the temperature of boiling water must destroy all living organisms was a mistaken one, and that, especially in alkaline liquids, a higher temperature was required to insure sterilization. His experiments showed that a temperature of 110° to 112° C. (230° to 233.6° F.), which he obtained by boiling under a pressure of one and a half atmospheres, was sufficient in every case. These experiments, which have been repeated by numerous investigators since, settled the spontaneous-generation controversy ; and it is now generally admitted that no development of microörganisms occurs in organic liquids, and no processes of putrefaction or fermentation occur in such liquids, when they are completely sterilized and guarded against the entrance of living germs from without.

Pasteur at a later date (1865) showed that the atmospheric organisms which resist the boiling temperature are in fact reproductive bodies, or *spores*, which he described under the name of "corpuscles ovoides" or "corpuscles brillants." Spores had been previously seen by Perty (1852) and Robin (1853), but it was not until 1876 that the development of these reproductive bodies was studied with care by Cohn and by Koch. The last-named observer determined the conditions under which spores are formed by the anthrax bacillus. Five years later (1881) Koch published his valuable researches relating to the resisting power of anthrax spores to heat and to chemical agents.

The development of our knowledge relating to the bacteria, stimulated by the controversy relating to spontaneous generation and by the demonstration that various processes of fermentation and putrefaction are due to microörganisms of this class, has depended largely upon improvements in methods of research. Among the most important points in the development of bacteriological technique we may mention, *first*, the use of a cotton air filter (Schröder and Von Dusch, 1854); *second*, the sterilization of culture fluids by heat (methods perfected by Pasteur, Koch, and others); *third*, the use of the aniline dyes as staining agents (first recommended by Weigert in 1877); *fourth*, the introduction of solid culture media, and the "plate method" for obtaining pure cultures, by Koch in 1881.

The various improvements in methods of research, and especially the introduction of solid culture media and Koch's "plate method" for isolating bacteria from mixed cultures, have placed bacteriology upon a scientific basis, and have shown that many of the observations and inferences of the earlier investigators were erroneous owing to the imperfection of the methods employed.

Since it has been demonstrated that certain infectious diseases of man and the lower animals are due to organisms of this class, physicians have been especially interested in bacteriological researches, and the progress made during the past fifteen years has been largely due to their investigations. It was a distinguished French physician, Davaine, who first demonstrated the etiological relation of a microörganism of this class to a specific infectious disease. The anthrax bacillus had been seen in the blood of animals dying from this disease by Pollender in 1849 and by Davaine in 1850, but it was several years later (1863) before the last-named observer claimed to have demonstrated by inoculation experiments the causal relation of the bacillus to the disease in question.

The experiments of Davaine were not generally accepted as conclusive, because in inoculating an animal with blood containing the bacillus, from an infected animal which had succumbed to the disease, the living microörganism was associated with material from the body of the diseased animal. This objection was subsequently removed by the experiments of Pasteur, Koch, and many others with pure cultures of the bacillus, which were shown to have the same pathogenic effects as had been obtained in inoculation experiments with the blood of an infected animal.

The next demonstration of the causal relation of a parasitic microörganism to an infectious malady was made by Pasteur, who devoted several years to the study of an infectious disease of silkworms which threatened to destroy the silk industry of France—*pébrine*.

In 1873 Obermeier, a German physician, announced the discovery, in the blood of patients suffering from relapsing fever, of a minute, spiral, actively motile microörganism—the *Spirochœte Obermeieri*—which is now generally recognized as the specific infectious agent in this disease.

The very important work of Koch upon traumatic infectious diseases was published in 1878.

In 1879 Hansen reported the discovery of bacilli in the cells of leprous tubercles, and subsequent researches have shown that this bacillus is constantly associated with leprosy and presumably bears an etiological relation to the disease.

In the same year (1879) Neisser discovered the "gonococcus" in gonorrhœal pus.

The bacillus of typhoid fever was first observed by Eberth, and independently by Koch, in 1880, but it was not until 1884 that Gaffky's important researches relating to this bacillus were published.

In 1880 Pasteur published his memoir upon fowl cholera, and the same year appeared several important communications from this pioneer in bacteriological research upon the "attenuation" of the virus of anthrax and of fowl cholera and upon protective inoculations in these diseases.

In 1880 the present writer discovered a pathogenic micrococcus, which he subsequently named *Micrococcus Pasteuri*, and which is now generally recognized as the usual agent in the production of acute croupous pneumonia—commonly spoken of as the "diplococcus pneumoniæ," but described in the present volume under the name of *Micrococcus pneumoniœ crouposœ.*

In 1881 several important papers by Koch and his colleagues appeared in the first volume of the "Mittheilungen" published by the Imperial Board of Health of Germany.

The following year (1882) Koch published his discovery of the tubercle bacillus.

The same year Pasteur published his researches upon the disease of swine, known in France as *rouget.*

The same investigator (Pasteur) also published in 1882 his first communication upon the subject of rabies.

Another important discovery was made in 1882 by the German physicians Löffler and Schütz, viz., that of the bacillus of glanders.

Koch published his discovery of the cholera spirillum—"comma bacillus"—in 1884.

The same year (1884) Löffler discovered the diphtheria bacillus.

Another important publication during the same year was that of Rosenbach, who, by the application of Koch's methods, fixed definitely the characters of the various microörganisms found in pus from acute abscesses, etc.

The tetanus bacillus was discovered in 1884 by Nicolaier, a student in the laboratory of Prof. Flügge, of Göttingen. That this bacillus is the cause of tetanus in man has been demonstrated by the subsequent researches of numerous investigators. For an exact knowledge of its biological characters we are especially indebted to Kitasato.

So far as human pathology is concerned, no important pathogenic microörganism was discovered after the year 1884 until the year 1892. After numerous unsuccessful researches by competent bacteriologists, a bacillus was discovered by Pfeiffer, of Berlin, and independently by Canon, which is believed to be the specific cause of influenza.

In 1894 the distinguished Japanese bacteriologist, Kitasato, during a visit to China made for the purpose, discovered the bacillus of the bubonic plague of the Orient.

Finally, we may refer to the discovery of the antitoxins of diphtheria and of tetanus as among the most important events in the history of bacteriology and of scientific medicine. The name of Behring has the first place in connection with this discovery.

Having briefly passed in review some of the principal events in the progress of our knowledge in this department of scientific investigation, it will be of interest to students to know something more of the literature of bacteriology. Important papers have appeared in medical and scientific journals in all countries, and research work of value has been done by enthusiastic investigators of nearly every nation. The brilliant pioneer work done by Pasteur and by Koch has attracted to them many pupils and has made France and Germany the leading countries in this line of investigation. The very great advantages of Koch's methods of research, introduced at the commencement of the last decade, have attracted many students from various parts of the world to Berlin, and to other cities of Germany where instruction was to be obtained from some of Koch's earlier pupils. But to-day bacteriological laboratories have been established in all parts of the world, and it is no longer necessary to go to Germany to obtain such instruction. The literature of the subject is. however, largely in the German and French languages. We can only refer here to such periodicals as are principally devoted to bacteriological research work.

The *Zeitschrift für Hygiene* has been published since 1886, and contains numerous valuable papers, contributed for the most part by the pupils of Koch and of Flügge, who are the editors of the journal.

The *Annales de l'Institut Pasteur* is a monthly journal which has been published since 1888. It is edited by Duclaux, and contains many important papers and reviews, as well as the statistics of the

Pasteur Institute relating to preventive inoculations against hydrophobia.

The *Annales de Micrographie* is a monthly journal, published in Paris. The principal editor is Miquel.

The *Centralblatt für Bakteriologie und Parasitenkunde* is a weekly journal which has been published by Gustav Fischer, of Jena, since 1887. The editors are Uhlworm, of Cassel; Löffler, at present professor at Greifswald; and Leuckart, professor at Leipzig.

The *Journal of Pathology and Bacteriology* is published monthly in Edinburgh and London. It dates from 1892.

A most important work for students of bacteriology is the *Jahresbericht* of Baumgarten, which has been published since 1885 by Harald Bruhn, Braunschweig, Germany. This gives a brief abstract of nearly every paper of importance relating to the subject which has been published during the year.

The *Journal of Hygiene* is a new quarterly, edited by Dr. George H. F. Nuttall, and published in Cambridge, England. In the first number (January 1st, 1901) the accomplished editor says: "The *Journal of Hygiene* will fulfil a definite purpose by serving as a focus to English-speaking investigators for work in Physics, Chemistry, Physiology, Pathology, Bacteriology, Parasitology, and Epidemiology, in relation to Hygiene and Preventive Medicine."

II.

CLASSIFICATION.

THE earlier naturalists—Ehrenberg (1838), Dujardin (1841)—placed the bacteria among the infusoria; but they are *now recognized as vegetable microörganisms*, differing essentially from the infusoria, which are unicellular animal organisms. One of the principal points in differentiating animal from vegetable organisms among the lowest orders of living things is the fact that animal organisms receive food particles into the interior of the body, assimilating the nutritious portion and subsequently extruding the non-nutritious residue ; vegetable organisms, on the other hand, are nourished through the cell wall which encloses their protoplasm, by organic or inorganic substances held in solution.

Ehrenberg (1838), under the name of vibrioniens, established four genera, as follows:

1. *Bacterium*—filaments linear and inflexible.
2. *Vibrio*—filaments linear, snake-like, flexible.
3. *Spirillum*—filaments spiral, inflexible.
4. *Spirochœte*—filaments spiral, flexible.

Dujardin (1841) united the two genera *Spirillum* and *Spirochœte* of Ehrenberg, and added to the description of the generic characters as follows:

1. *Bacterium*—filaments rigid, with a vacillating movement.
2. *Vibrio*—filaments flexible, with an undulatory movement.
3. *Spirillum*—filaments spiral, movement rotatory.

It will be seen that this classification leaves no place for the motionless bacilli, such as the anthrax bacillus and many others, and does not include the spherical bacteria, now called micrococci.

The classification of Davaine (1868) provides for the motionless, filamentous bacteria, but does not include the micrococci. This author first insisted that the vibrioniens of Ehrenberg are truly vegetable organisms, allied to the algæ. He makes four genera, as follows:

Filaments straight or bent, but not in a spiral,	Moving spontaneously,	Rigid *Bacterium*. Flexible *Vibrio*.
	Motionless, .	*Bacteridium*.
Filaments spiral, ...		*Spirillum*.

Following Davaine, the French bacteriologists frequently speak of the motionless anthrax bacillus as *la bactéridie*.

Hoffman in 1869 included in his classification the spherical bacteria, and pointed out the fact that motility could not be taken as a generic character, as it was not constant in the same species and depended to some extent upon temperature conditions, etc.

Having determined that the bacteria are truly vegetable organisms, the attention of botanists has been given to the question as to what class of vegetable organisms they are most nearly related to. There are decided differences of opinion in this regard. While Davaine, Rabenhorst, and Cohn insist upon their affinities with the algæ, Robin, Nägeli, and others consider them fungi. One of the principal characters which distinguish the algæ from the fungi is the presence of chlorophyll in the former and its absence in the latter. Now, the bacteria are destitute of chlorophyll, and in this regard resemble the fungi; yet in others their affinities with the *Palmellaceæ* and *Oscillatoriaceæ* are unmistakable. It is not necessary, however, that we should consider them as belonging to either of these classes of the vegetable kingdom. By considering them a distinct class of unicellular vegetable organisms, under the general name of bacteria, we may avoid the difficulties into which the botanists have fallen.

We must refer briefly, however, to the classification proposed by some of the leading German botanists.

Nägeli, placing the bacteria among the lower fungi, which give rise to the decomposition of organic substances, divides these into three groups:

1. The *Mucorini*, or mould fungi.
2. The *Saccharomycetes*, or budding fungi, which produce alcoholic fermentation in saccharine liquids.
3. The *Schizomycetes*, or fission fungi, which produce putrefactive processes, etc.

Cohn, under the name of *Schizophytes*, has grouped these low vegetable organisms, whether provided or not with chlorophyll, into two tribes having the following characters:

1. GLÆOGENES—cells free or united into glairy families by an intercellular substance.
2. NEMATOGENES—cells disposed in filaments.

In the first tribe he has placed the genera *Micrococcus* (Hallier), *Bacterium* (Dujardin), *Merismopedia* (Meyer), *Sarcina* (Goodsir), and *Ascococcus* (Billroth), with various genera of unicellular algæ containing chlorophyll.

In the second tribe we have the genera *Bacillus* (Cohn), *Leptothrix* (Kg.), *Vibrio* (Ehr.), *Spirillum* (Ehr.), *Spirochœte* (Ehr.), *Streptococcus* (Billr.), *Cladothrix* (Cohn), and *Streptothrix* (Cohn), associated with genera of green filamentous algæ.

The German botanist Sachs unites the fungi and the algæ into a single group, the *Thallophytes*, in which he establishes two parallel series, one including those containing chlorophyll, and the other without, as follows:

THALLOPHYTES.

Forms with chlorophyll.	Forms without chlorophyll.
Class I.—Protophytes.	
A. Cyanophyceæ (Oscillatoriaceæ, etc.).	*A.* Schizomycetes (Bacteria).
B. Palmellaceæ.	*B.* Saccharomycetes.

Zopf, who insists upon the polymorphism of these low organisms, divides the bacteria into four groups:

	Genera.
1. Coccoceæ —Up to the present time, only known in the form of cocci.	*Streptococcus*, *Merismopedia*, *Sarcina*, *Micrococcus*, *Ascococcus*.
2. Bacteriaceæ.—Have for the most part spherical, rod-like, and filamentous forms; the first (cocci) may be wanting; the last are not different at the two extremities; filaments straight or spiral.	*Bacterium*, *Spirillum*, *Vibrio*, *Leuconostoc*, *Bacillus*, *Clostridium*.
3. Leptotricheæ. — Spherical, rod-shaped, and filamentous forms; the last show a difference between the two extremities; filaments straight or spiral; spore formation not known.	*Crenothrix*, *Beggiatoa*, *Phragmidiothrix*, *Leptothrix*.
4. Cladotricheæ. — Spherical, rod-shaped, filamentous, and spiral forms; the filamentous form presents pseudo-branches; spore formation not known.	*Cladothrix*.

The main objection to this classification is that it assumes a pleomorphism for the bacteria of the second group—Bacteriaceæ—which has only been established for a few species, and which appears not to be general among the rod shaped and spiral bacteria.

De Bary divides the bacteria into two principal groups, one including those which form endospores, and the other those which are reproduced by arthrospores. But our knowledge is yet too imperfect to make this classification of value, and the same may be said of Hueppe's recent attempt at classification, in which the mode of reproduction is a principal feature.

The classification of Baumgarten (1890) appears to us to have more practical value, and, with slight modifications, we shall adopt it in the present volume. This author divides the bacteria into two principal groups, as follows:

Group I. Species relatively monomorphous.

Group II. Species pleomorphous.

The first group includes the *micrococci*, the *bacilli*, and the *spirilla;* the second group the *spirulina* of Hueppe, *leptotricheæ* (Zopf), and *cladotricheæ*.

The pleomorphous species described by Hauser under the generic name Proteus are included in the second group among the spirulina. In the present volume we have described these pleomorphous species among the bacilli.

The Cocci, in the classification of Baumgarten, constitute a single genus with the following subgenera: 1, *Diplococcus;* 2, *Streptococcus;* 3, *Merismopedia* (Zopf)—"Merista" (Hueppe); 4, *Sarcina* (Goodsir); 5, *Micrococcus* ("staphylococci").

The Bacilli are included in a single genus embracing all of those species which only form rod-shaped cells, and filaments composed of rod-like segments; or straight filaments not distinctly segmented, which may be rigid or flexible.

The SPIRILLA are also included in a single genus, embracing all of those species in which the filaments are spiral in form and the segments more or less spiral or "comma-shaped"—filaments either rigid or flexible.

This simple morphological classification of the monomorphous group of Baumgarten corresponds with the nomenclature now generally in use among bacteriologists. We speak of the spherical bacteria as *cocci* or as *micrococci*, of the rod-shaped bacteria as *bacilli*, and of the spiral bacteria as *spirilla*.

It is true, however, that we are sometimes embarrassed to decide whether a particular microörganism belongs to one or the other of these morphological groups or so-called genera. Among the bacilli, for example, we may have, in the same pure culture, rods of very different lengths, some being so short that if alone they might be taken for cocci, while others may have grown out into long filaments. But if we are assured that the culture is pure the presence of rod forms establishes the diagnosis, and usually the cocci-like elements, when carefully observed, will be seen to be somewhat longer in one diameter than in the other. The German bacteriologists generally insist upon placing among the bacilli all straight bacteria in which, as a rule, one diameter is perceptibly greater than that transverse to it; and several species of well-known bacteria which were formerly classed as micrococci are now called bacilli—*e.g.*, Friedländer's bacillus ("pneumococcus"), *Bacillus prodigiosus*.

The distinction made by Cohn and others between the genus *Bacterium* (Duj.) and the genus *Bacillus* (Cohn) cannot be maintained, inasmuch as we may have short rods and quite long filaments in the same pure culture of a single species; and, again, the character upon which the genus *Vibrio* (Ehr.) was established—viz., the fact that the filaments are flexible and the movements sinuous—is not a sufficient generic or even specific character, for in a pure culture there may be short rods which are rigid, and long filaments which are flexible and have a sinuous movement. We therefore to-day speak of all the elongated forms as bacilli, unless they are spiral and have a corkscrew-like motion, in which case they are known as spirilla.

The bacteria are also classified according to their biological characters, and it will be necessary to consider the various modes of grouping them from different points of view other than that which relates to their form. This is the more important inasmuch as we are not able to differentiate species by morphological characters alone. Thus, for example, there are among the spherical bacteria, or micrococci, numerous well-established species which the most expert microscopist could not differentiate by the use of the microscope alone; the same is true of the rod-shaped bacteria. The assumption often made by investigators who are not sufficiently impressed with this fact, that two microörganisms from different sources, or even from the same source, are the same because stained preparations examined under the microscope look alike, has led to serious errors and to much confusion. As an example of what is meant we may refer to the pus organisms. Before the introduction of Koch's "plate method" micrococci had been observed in the pus of acute abscesses. Some of these were grouped in chains—streptococci—and some were single, or in pairs, or in groups of four; but whether these were simply different modes of grouping in a single species, or whether the chain micrococci represented a distinct species, was not determined with certainty. That there were in fact four or more distinct species to be found in the pus of acute abscesses was not suspected until Rosenbach and Passet demonstrated that this is the case, and showed that not only is the streptococcus a distinct species, but that among the cocci not associated in chains there are three species which are to be distinguished from each other by their color when grown on the surface of a solid culture medium. One of these has a milk-white color, one is of a lemon-yellow color, while the third is a golden-yellow.

Those microörganisms which form pigment are called *chromogenes*, or chromogenic; those which produce fermentations are spoken of as *zymogenes*, or zymogenic; those which give rise to disease processes in man or the lower animals are denominated *pathogenes*, or pathogenic. We cannot, however, classify bacteria under the three headings chromogenes, zymogenes, and pathogenes, for some of the chromogenic species are also pathogenic, as are some of the zymogenes. These characters must therefore be considered separately as regards each species, and in studying its life history and distinguishing characters we determine whether it is chromogenic or non-chromogenic; whether it produces special fermentations; and whether it is or is not pathogenic when inoculated into the lower animals. In making the distinction between pathogenic and non-pathogenic microörganisms we must remember that a certain species may be pathogenic for one animal and not for another. Thus the anthrax bacillus, which is fatal to cattle, sheep, rabbits, guinea-pigs, and mice, does not kill white rats; the bacillus of mouse septicæmia kills house mice, but field mice are fully immune from its pathogenic effects; on the other hand, the bacillus of glanders is fatal to field mice but not to house mice.

✓ Again, it must be remembered that pathogenic power also depends, to a greater or less extent, upon the dose injected into an animal as compared to its body weight. Some pathogenic organisms in very minute doses give rise to a fatal infectious malady; others are only able to overcome the vital resisting power of the tissues and fluids of the body when introduced into the circulation, or into the subcutaneous tissue or abdominal cavity, in considerable amounts. Some pathogenic bacteria invade the blood; others multiply only in certain tissues of the body; and others again multiply in the intestine and by the formation of poisonous products which are absorbed show their pathogenic power.

Another classification of the bacteria relates to the environment favorable to their development. Thus we speak of saprophytic and parasitic bacteria, or of SAPROPHYTES and PARASITES.

The *saprophytes* are such as exist independently of a living host, obtaining their supply of nutriment from dead animal or vegetable material and from water containing organic and inorganic matters in solution. The *strict parasites*, on the other hand, depend upon a living host, in the body of which they multiply, sometimes without injury to the animal upon which they depend for their existence, but frequently as harmful invaders giving rise to acute or chronic infectious diseases. Microörganisms which ordinarily lead a saprophytic existence, but which can also thrive within the body of a living animal, are called *facultative parasites.* Thus the leprosy bacillus, which is only found in leprous tissues, is a strict parasite; while the typhoid bacillus, the cholera spirillum, etc., are facultative parasites, inasmuch as they are capable of maintaining an independent existence, for a time at least, external to the bodies of living animals.

It seems probable that the pathogenic organisms which are only known to us to-day as strict parasites were, at some time in the past, saprophytes, which gradually became accustomed to a parasitic mode of existence, and, under the changed conditions of their environment, finally lost the power of living in association with other saprophytes exposed to variations of temperature, etc. The tubercle bacillus, for example, is known to us only as a parasite which has its habitat in the lungs, lymphatic glands, etc., of man and of certain of the lower animals. But we are able to cultivate it in artificial media external to the body; and it is in accord with modern views relating to the development of species to suppose that at some time in the past it was able to lead a saprophytic existence. Not to admit this forces us to the conclusion that, at some time subsequent to the appearance of man and the lower animals in which it is now found as a parasite, it was created with its present biological characters, which restrict it to a parasitic existence in the bodies of these animals, and that, consequently, the immense destruction of human life which has resulted from its parasitic invasion of successive generations was designed when it was created. The opposite view is supported by numerous facts which show that these low organisms, like those higher in the scale, are subject to modifications as a result of changed conditions of environment, and that such modifications, in the course of time, may become well-established specific characters.

Again, the bacteria may be grouped into *aërobic* and *anaërobic* species. This is a very important distinction, which was first established by Pasteur, who found that certain bacteria will only grow when freely supplied with oxygen, while others absolutely decline to grow in the presence of this gas. The latter, which are spoken of as *strict anaërobics,* may be cultivated in a vacuum or in an atmosphere of hydrogen. Those species which grow either in the presence of oxygen or when it is excluded are called *facultative anaërobics.*

Certain bacteria produce a peptonizing ferment which has the power of liquefying gelatin. This has led to the classification of those microörganisms of this class which grow in Koch's flesh-peptone-gelatin as *liquefying* and *non-liquefying* bacteria.

Again, we speak of them as *motile* or *non-motile.*

It is evident that these biological characters, although all-important in the definition of species, cannot serve us in an attempt to establish natural genera ; for the lines are not sharply drawn between the saprophytes and the parasites, the aërobics and the anaërobics, etc., inasmuch as we have facultative parasites and facultative anaërobics which we cannot include in either class, and which yet do not form a distinct class by themselves. We therefore adhere to the morphological classification, although this is open to criticism. For example, among the rod-shaped organisms which we call bacilli and describe under the generic name *Bacillus* there are some which multiply by binary division only, while others form endogenous reproductive bodies known as *spores.* Certainly so important a difference in the mode of reproduction should be sufficient to separate these rod-shaped organisms into two natural groups or genera.

As heretofore stated, the German bacteriologist Hueppe has attempted a classification based upon the mode of reproduction, in which he makes two groups, or "tribes," one in which reproduction occurs by the formation of endogenous spores—"*endospores*"—the other in which it occurs by the formation of "*arthrospores.*"[1] The latter group includes all of those bacteria in which no other mode of multiplication is known than that by binary division, which is common to all. In the present state of our knowledge this classification is scarcely to be considered of practical value, inasmuch as the question of spore formation is still undetermined for a large number of species.

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[1] An account of this mode of reproduction is given on page 19.

In the following table we shall give the characters of the different genera which have been described by recent botanists and bacteriologists, arranged under the three headings, MICROCOCCI, BACILLI, SPIRILLA. Where we doubt the propriety of maintaining a distinct generic name upon the supposed distinguishing characters, the description will be printed in small type.

MICROCOCCI.

General Characters.—Spherical bacteria which are reproduced by binary division; usually without spontaneous movements; do not form endogenous spores. (According to some authors, certain cells, known as arthrospores, may be distinguished by their greater size and refractive power, and these are supposed to have greater resistance to desiccation than the ordinary cocci resulting from binary division, and to serve as reproductive bodies.) Some micrococci are not precisely round, but are somewhat oval in form; and when in process of division the cocci, necessarily, are more or less elongated in one diameter before a complete separation into two spherical elements has occurred.

MICROCOCCUS.—Division in one direction; cocci single, in pairs, or accidentally associated in irregular groups; sometimes held together in irregular masses by a transparent, glutinous, intercellular substance. (Micrococci belonging to this genus are frequently described as "staphylococci," and Staphylococcus is used by Rosenbach as a generic name for the pus cocci described by him, which are solitary or associated in irregular groups, as above described.)

ASCOCOCCUS.—Cocci associated in globular or lobulated, zoöglœa masses by a rather firm intercellular substance.

LEUCONOSTOC.—Cocci, solitary or in chains, surrounded by a thick, gelatinous envelope and forming zoöglœa of cartilaginous consistence.

STREPTOCOCCUS.—Division in one direction only; cocci associated in chains.

Diplococcus.—Division in one direction only; cocci associated in pairs. Association in pairs is common to all of the micrococci, inasmuch as they multiply by binary division. When such association has rather a permanent character, it is customary to speak of the microörganism as a diplococcus, but we doubt the propriety of recognizing this mode of association as a generic character.

MERISMOPEDIA.—Division in two directions, forming groups of four, which remain associated in a single plane—"tetrads."

SARCINA.—Division in three directions, forming packets of eight or more elements, which remain associated in more or less regular cubical masses.

BACILLI.

General Characters.—Rod-shaped and filamentous (not spiral) bacteria in which there is no differentiation between the extremities of the rods; reproduction by binary division in a direction transverse to the long axis of the rods, or by binary division and the formation of endogenous spores; rigid or flexible; motile or non-motile.

BACILLUS.—Characters as given above.

Bacterium.—This genus, established by Dujardin, is now generally abandoned, the species formerly included in it being transferred to the genus *Bacillus.* As defined by Cohn, the generic characters were: Cells cylindrical or elliptical, free or united in pairs during their division, rarely in fours, never in chains, sometimes in zoöglœa (differing from the zoöglœa of spherical bacteria by a more abundant and firmer intercelluar substance), having spontaneous movements, oscillatory and very active, especially in media rich in alimentary material and in presence of oxygen.

Clostridium.—Rod-shaped bacteria which form large, endogenous, and usually oval spores; these are centrally located, and during the stage of spore formation the rods become fusiform.

SPIRILLA.

General Characters.—Curved rods or spiral filaments; rigid or flexible; reproduction by binary division, or by binary division and the formation of endogenous spores (or by arthrospores?); movements rotatory in the direction of the long axis of the filaments.

SPIRILLUM.—Characters as above.

Spirochœte.—Flexible, spiral filaments; movements rotatory.

Vibrio.—Filaments flexible, straight or sinuous; movements sinuous.

A considerable number of bacteria which are usually seen as short, curved rods, but which may grow out into long, spiral filaments, are described by some authors under the generic name Vibrio, *e.g.*, the so-called "comma bacillus" of Koch—"Spirillum choleræ Asiaticæ"; the spirillum of Finkler and Prior—"Vibrio proteus"; the spirillum described by Gameléia—"Vibrio Metschnikovi," etc. These microörganisms have not the characters which distinguished the genus Vibrio as established by Ehrenberg, and we prefer to follow Flügge in describing them under the generic name Spirillum.

The pathogenic bacteria now known belong to one or the other of the above-described genera, and the attention of bacteriologists has been given chiefly to the study of micrococci, bacilli, and spirilla. But the botanists place among the bacteria certain other forms which are found in water, and which, in a systematic account of this class of microörganisms, demand brief attention at least. These are included in Baumgarten's second group, which includes the pleomorphous bacteria.

SPIRULINA (Hueppe).—The vegetative cells are sometimes rod-shaped and sometimes spiral; in suitable media they may grow out into long, straight, wavy, or spiral filaments. These filaments may break up into cocci-like reproductive elements—"arthrospores."

LEPTOTRICHEÆ (Zopf).—The vegetative cells present rod-shaped and spiral forms, and grow out into straight, wavy, or spiral filaments; these may show a difference between the two extremities, of base and apex. Cocci-like reproductive bodies are formed by segmentation of the rod-shaped elements in these filaments. In some of the species the segments are enclosed in a common sheath. *Subgenera:* LEPTOTHRIX, BEGGIATOA, CRENOTHRIX, PHRAGMIDIOTHRIX (for generic characters see page 12).

CLADOTRICHEÆ (Zopf).—The vegetative cells are rod-shaped or spiral, and grow out into straight or spiral filaments, which may present pseudo-ramifications. A single genus, CLADOTHRIX (see page 12).

The various methods of classification heretofore referred to must all be considered provisional and unsatisfactory from a scientific point of view. Thus Hueppe says: "The existence of rigid form species, which not only the earlier observers, but even Cohn, Schröter, and Koch assumed, can be upheld no longer. The adaptability of bacterial forms to changing conditions of nutrition is not so boundless as Naegeli and Billroth supposed, but it is considerably greater than was once held to be compatible with the conception of the existence of constant species."

A. Fischer has attempted to make use of the presence, number, and mode of attachment of flagella as a means of classification. No doubt this character and the presence or absence of spores should receive consideration in any attempt at a scientific classification of the bacteria.

III.

MORPHOLOGY.

In the present chapter we shall give a general account of the morphology, modes of grouping, and dimensions of the bacteria.

The standard of measurement used by bacteriologists is the micromillimetre, or the one-thousandth part of a millimetre. This is represented by the Greek letter μ. One μ (micromillimetre) is equal to about one-twenty-five-thousandth of an English inch.

The spherical bacteria, or micrococci, differ greatly in size, and also in the mode of grouping when, as a result of binary division, they remain associated one with another. The smallest may measure no more than 0.1μ, while some of the larger species are from one to two μ in diameter. The enormous number of these minute organisms which may be contained in a small drop of a pure culture may be easily estimated in a rough way. Compare a single micrococcus, for example, with a sphere having a diameter of one-twenty-fifth of an inch. If our micrococcus is one of the larger sort, having a diameter of one μ, it would take a chain of one thousand to reach across the diameter of such a sphere, and its mass, as compared to the larger sphere, would be as 1 to 523,600,000.

The number of cocci in a milligramme of a pure culture of Staphylococcus pyogenes aureus has been estimated by Bujwid, by counting, at 8,000,000,000.

Not only do different species differ in dimensions, but considerable differences in size may be recognized in the individual cocci in a pure culture of the same species. On the other hand, there are numerous species which so closely resemble each other in size and mode of association that they cannot be differentiated by a microscopic examination alone, and we must depend upon other characters, such as color, growth in various culture media, pathogenic power, etc., to decide the question of identity or non-identity.

When in active growth the micrococci necessarily depart from a typical spherical form just before dividing, and under these circumstances may be of a short or long oval. When division has taken place, if the two members of a pair remain associated they are often more or less flattened at the point of contact (Fig. 1, *a*).

When in a culture the cocci are for the most part associated in pairs (Fig. 1, *d*), we speak of the organism as a *diplococcus*.

The *staphylococci* are characterized by the fact that, for the most part, the individual cocci in a culture are solitary (Fig. 1, *b*). But, inasmuch as multiplication occurs by binary division, we also have pairs and occasionally a group of four—probably from the accidental apposition of two pairs (Fig. 1, *c*); or they may be associated in grapelike bunches; and after staining and mounting a preparation we find the cells associated in irregular groups. This results from the fact that they are surrounded by a glutinous material which causes them to adhere to each other (Fig. 1, *e*). A mass of cocci held together in this way by a transparent, glutinous, intercellular substance is spoken of as a *zoöglœa* (Fig. 2). In the genus *Ascococcus* the intercellar substance is quite firm and the zoöglœa are in the form of spherical or irregularly lobulated masses surrounded by a resistant envelope of jelly-like material (Fig. 3).

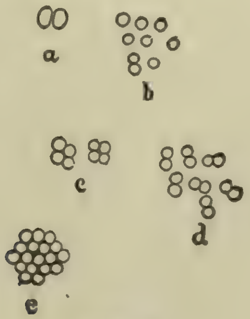

FIG. 1.

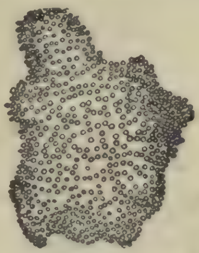

FIG. 2.

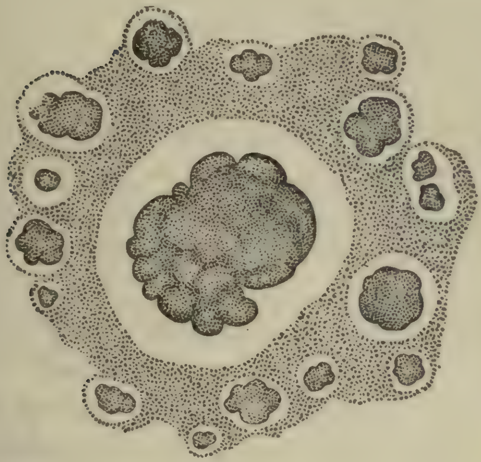

FIG. 3.

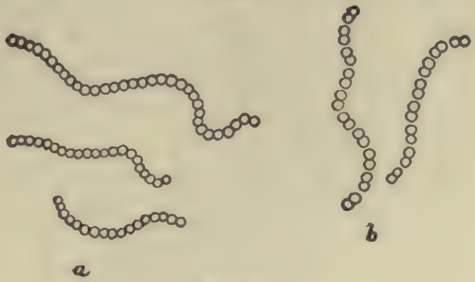

FIG. 4.

When, as a result of division in one direction only, the cocci remain united in chains (Fig. 4, *a*), they are described as *streptococci*, and are sometimes spoken of as in *chaplets* or in *torula chains*. In such chains we frequently find the evidence of recent division of the cocci, as shown by the grouping of the elements of the chain into pairs (Fig. 4, *b*).

When division occurs habitually in two directions, groups of four result, which are spoken of as *tetrads*. This is the distinguishing character of the genus *Merismopedia*. In these groups of four the individual cocci are often flattened at the points of contact, as in Fig. 5, *b*. We also find pairs and groups of three in pure cultures of species belonging to this genus, as shown in Fig. 5, *c*. In these, transverse division has not yet occurred in one or in both elements of a pair. This association of micrococci in tetrads seems to be maintained, in some species at least, by the fact that each group of four is enclosed in a jelly-like *capsule*. The extent of this capsule differs in the same species under different circumstances; as a rule, it is most apparent when a culture has been made in a liquid medium. Some of the diplococci have a similar capsule. The jelly-like substance does not stain well with the aniline colors and is seen as a transparent halo around the stained cocci. Some authors (Fränkel and Pfeiffer) believe that this capsule is formed by the swelling up of the cell membrane as a result of the imbibition of water.

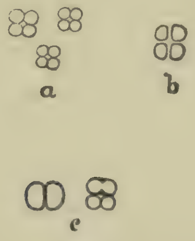

FIG. 5.

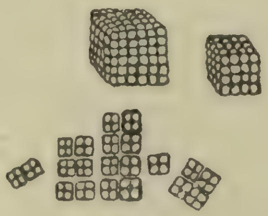

FIG. 6.

When division occurs in three directions packets of eight or more elements are formed. This mode of association characterizes the genus *Sarcina*. The "packet form" is best seen in an unstained preparation from a fresh culture, in which a little material suspended in water is examined under a comparatively low-power objective—one-sixth (Fig. 6).

Among the bacilli there is room for a wider range of morphological characters. They differ not only in dimensions and in modes of grouping, but in form. The relation of the transverse to the longitudinal diameters affords a great variety of forms, varying from a short oval element to a slender rod or elongated filament. But it must be remembered that we may have short rods and long filaments in a pure culture of the same bacillus—the typhoid bacillus, for example. There are also considerable differences in the transverse diameter of bacilli belonging to the same species when cultivated in different media, or even in the same medium, although, as a rule, the transverse diameter is tolerably uniform in pure cultures.

Again, the form of the extremities of the rods is to be observed (Fig. 7). This may be square, or the corners may be slightly rounded, or the extremities may be quite round or lance-oval, or the outlines of the rod may be spindle-shaped from the formation of a large central spore—"*clostridium*"—or one end may be dilated from the formation of a large terminal spore.

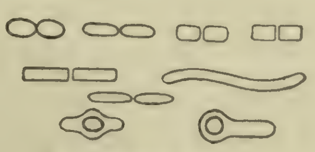

FIG. 7.

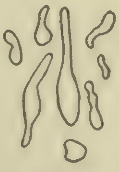

FIG. 8.

In old cultures we frequently find irregular forms due to swellings and constrictions, which probably occur in bacilli which have but little vitality or are already dead. These are spoken of as *involution forms* (Fig. 8).

The bacilli multiply by binary division in a direction transverse to the longitudinal axis, and, as a result of such binary division, long chains in which the elements remain associated may be formed (Fig. 9); or the rods may be for the most part solitary or united in pairs. Like the micrococci, the bacilli are sometimes surrounded by a gelatinous envelope or capsule. They may also be united by a glutinous material into zoöglœa masses.

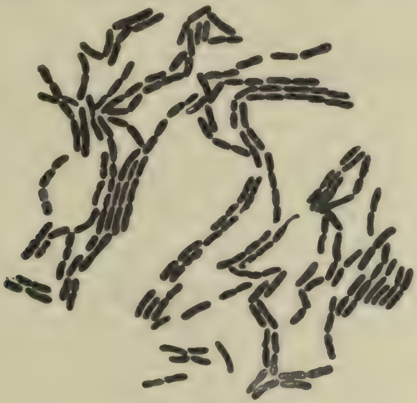

FIG. 9.

Bacilli which under certain conditions are seen as short rods may, under other circumstances, grow out into long filaments, and these may be associated in bundles or in tangled masses.

The spirilla differ from the bacilli in the form of the rods and filaments, which are curved or spiral. The shorter elements in a pure culture may be simply curved, as in *a*, Fig. 10, while the spiral form becomes apparent in those which are longer, and we may have one or several turns of the spiral (Fig. 10, *b*). The spiral form may be but slightly marked (Fig. 10, *c*), or the turns may be close and deep as in a corkscrew (Fig. 10, *d*). Again, the curved filaments may be short and rigid, or long and flexible (Fig. 10, *e*).

In the genus *Cladothrix,* which is placed by botanists among the bacteria, the filaments appear to branch; but this branching is only apparent, and there is no true dichotomous branching in this class of microörganisms. The false branching of *Cladothrix dichotoma*, Cohn, is shown in Fig. 11. The fact that some of the larger species of bacilli and spirilla are provided with slender, whip-like appendages called *flagella* has been known for many years, and it has for some time been suspected that all of the motile organisms of this class are provided with similar appendages and that these are organs of locomotion. Recently, by improvements in methods of staining, Löffler has demonstrated the presence of flagella in many species in which they had heretofore escaped observation. They are sometimes single, at the ends of the rods (Fig. 12, *a*); or there may be several at the extremity of a single rod (Fig. 12, *b*); again, they are seen in considerable numbers around the periphery of the rod (Fig. 12, *c*).

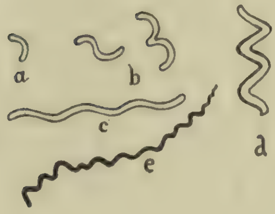

FIG. 10.

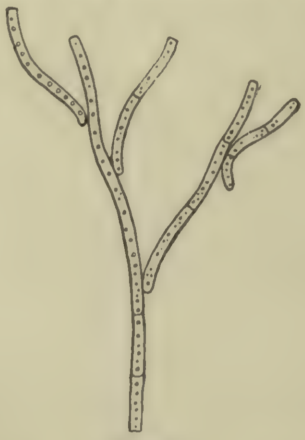

FIG. 11.

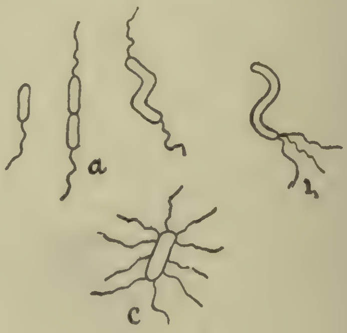

FIG. 12.

The bacilli and spirilla sometimes contain in the interior of the cells granules of different kinds. These may appear like little oil drops or they may be more opaque. In the genus *Beggiatoa* grains of sulphur are found in the interior of the cells. Again, we may find vacuoles in the protoplasm; or, in stained preparations, deeply stained granules, which are not spores, may be seen at the extremities of the rods—end-staining. The morphological characters depending upon the formation of endogenous spores will be referred to hereafter.

IV.

STAINING METHODS.

THE rapid development of our knowledge with reference to the minute microörganisms under consideration depends very largely upon the discovery that they may be stained by various dyes, and especially by the aniline colors. Weigert (1876) was the first to employ these colors in studying the bacteria, and Koch at once recognized the value of the method and made use of it in his researches.

The basic aniline colors are those employed, and among these the most useful are fuchsin, methylene blue, gentian violet, Bismarck brown, and vesuvin.

Staining upon the Cover Glass or Slide.—By a "cover-glass preparation" we mean that material supposed to contain bacteria has been spread out upon a thin glass cover, dried, and stained for microscopical examination. A small drop of a liquid culture may, for example, be spread upon a perfectly clean cover glass by means of a platinum wire held in a glass handle (Fig. 13). Or we may place a drop of water in the centre of the thin glass cover, and by means of the same instrument take a little material from a culture made upon the surface of a solid medium and distribute it through the drop. In this case we must be careful to take very little of the material, as the smallest quantity will contain an immense number of bacteria, and for a satisfactory view of the individual cells it is necessary that they be well separated from each other, in some parts of the preparation at least, and not massed together.

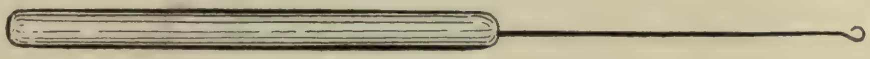

FIG. 13.

Where the object is to make a cabinet preparation for permanent preservation, special care should be taken to distribute the bacteria uniformly through the drop of water. The next step consists in evaporating the liquid so that the bacteria may remain attached to the surface of the glass cover. This may be done by simple exposure to the air or by the application of gentle heat. When the bacteria are suspended in an albuminous medium it will be necessary, after the film is dry, to heat the preparation sufficiently to coagulate the albumen, in order that it may not be washed off in the subsequent staining process. This is best done, in accordance with Koch's directions for the preparation of tuberculous sputum, by passing the cover glass, held in slender forceps, rather quickly through the flame of an alcohol lamp three times in succession. In this operation it must be remembered that too much heat will destroy the preparation, while too little will fail to accomplish the object in view—coagulation of the albumen. In passing the cover glass through the flame the smeared side is to be held upward. The time required will be about three seconds for passing it three times as directed; but this will vary according to the intensity of the flame, and some little experience is necessary in order to obtain the best results.

The operation of "fixing," or coagulating the albumen, may also be effected by exposure in a dry-air oven, heated to 120° to 130° C., for a few minutes (two to ten minutes), as directed by Ehrlich.

Bacteria simply suspended in distilled water adhere very well to the cover glass when treated as directed, but if they have been taken from a liquefied gelatin culture the film is very apt to be washed away during the staining process. This is best avoided by taking as little as possible of the gelatin medium and suspending the bacteria to be examined in a drop of water, which dilutes the gelatin and washes it away from the surface of the cells.

Smear Preparations.—In various infectious diseases bacteria are found in the blood and tissues of the body, and their presence may be demonstrated by making what is called a smear preparation. A little drop of blood may be spread upon the thin glass cover, or it may be brought in contact with the freshly cut surface of one of the vascular organs, as the liver or spleen. It is especially desirable that the material used for such a preparation be small in amount and distributed evenly in a very thin layer. In Germany it is the custom, in making smear preparations, to press the material between two glass covers, which are then separated by sliding them apart, thus leaving a thin layer upon each. This answers very well, but the writer prefers to spread the material by drawing across the face of the cover glass the end of a well-ground and polished glass slide. This method is especially useful for spreading blood in a uniform layer, in which the corpuscles are evenly distributed and retain their normal form. A very small drop of blood is placed near one edge of the cover glass, which is placed upon a smooth surface; the glass slide is held at a very acute angle and is gently drawn across the cover glass, without any pressure.

Most bacteriologists make their preparations upon the cover glass, as above described, but the writer has for a number of years made his mounts of bacteria upon the glass slide, and believes that this method has some advantages for every-day work. The thin glass covers required when a preparation is to be examined with an immersion objective of high power, are easily broken and often dropped from the fingers or forceps. When the material to be examined is spread and dried directly upon the glass slide, the operation is attended with less difficulty and fewer accidents and the results are quite as good. In this case the slide is held in the fingers during the various steps in the operation of distributing, drying, and staining, while the thin glass cover must be held in delicate forceps.

Contact Preparations.—When a dry and clean cover glass is brought in contact with a colony or surface culture we may often obtain a very pretty preparation, showing the bacteria in a single layer, and preserving the arrangement, as regards growth, which characterizes the species. Similar preparations may sometimes be obtained from the surface of liquid cultures, when the bacteria grow upon the surface as a thin film. The cover glass is to be gently brought into contact with this surface growth, which adheres to it and is dried and stained by the usual methods.

Staining of the dried film is quickly effected by using an aqueous solution of one of the aniline colors above mentioned. For general use the writer prefers a solution of fuchsin, on account of the promptness of its staining action, and because, in preparations for permanent preservation, it is not as likely to fade as methylene blue or gentian violet. It is also a better color than blue or violet in case a photomicrograph is to be made from the preparation.

It is best to keep on hand saturated alcoholic solutions of the staining agents named, and to make an aqueous solution whenever required by the addition of a few drops to a little water in a watch glass or test tube ; for the aqueous solutions do not keep well on account of the precipitation of the dye as a fine powder, which renders the solution opaque. The addition of ten per cent of alcohol to the aqueous solution will, however, prevent this precipitation ; but, as a rule, freshly prepared solutions are the best. These should be filtered before use. We may place a few drops of the filtered solution upon the dried film on the slide or cover glass, or the thin cover may be floated upon a little of the solution in a watch glass. In some cases it is best to use heat to expedite the staining, and this may be done by holding the slide or the watch glass over the flame of an alcohol lamp until steam commences to be given off. If the heating is carried too far the preparation is likely to be spoiled by the precipitation of the staining agent. As a rule, heating will not be necessary, and when an aqueous solution of fuchsin (one part to one hundred of water) is used most bacteria are stained within a few seconds to a minute. At the end of this time the staining solution is to be washed away by means of a gentle stream of water, or by moving the cover glass about in a vessel containing distilled water.

Decolorization.—It often happens that the albuminous material associated with the bacteria which we propose to examine is stained so deeply as to obscure the view of these; and, generally, we will obtain more satisfactory preparations by the use of a decolorizing agent, by which the background is cleared up and the outlines of the cells more clearly defined. The agents chiefly used for this purpose are alcohol, diluted acids, and solution of iodine with potassium iodide (Gram's solution).

Koch recommends a solution containing sixty parts of alcohol to forty parts of water. The cover glass is to be quickly passed through this solution two or three times. Some bacteriologists prefer to use absolute alcohol.

Or we may use dilute acetic acid (one-half to one per cent) or very dilute hydrochloric acid (ten drops to half a litre of water).

For decolorizing preparations containing the tubercle bacillus strong solutions of the mineral acids are employed (one part of nitric or of sulphuric acid to three parts of water).

Gram's solution contains one part of iodine and two parts of potassic iodide in three hundred parts of water. Special directions will be given for the use of these agents when we give an account of the staining methods most useful for the various pathogenic organisms.

Double Staining.—After decolorizing the background of albuminous material we may again stain this with a contrast stain, such as eosin or vesuvin. In mounts made from pure cultures, either liquid or solid, a single stain, for the bacteria only, is all that we require, and our aim is to have the background as free as possible from any material which would obscure the view.

After staining, decolorizing, and washing the preparation the cover glass or slide is again dried by exposure to the air or gentle heat, and is then ready for the permanent mounting in Canada balsam. If the bacteria have been stained upon the slide, a small drop of balsam dissolved in xylol is placed in the middle of the preparation and a clean, thin glass cover applied.

If it is the intention to make the microscopical examination with an immersion objective of high power, or to make photomicrographs from it, only the thinnest glass covers should be used—one-two-hundredths of an inch or less.

If the preparation is not intended for permanent preservation, the examination may be made without drying the surface upon which the stained bacteria are spread, the water taking the place of balsam in a permanent mount; or we may dry the film and use a drop of cedar oil between the slide and cover.

While simple aqueous solutions of the aniline colors, when freshly prepared, will promptly stain most bacteria, certain agents may be added to these which aid in the preservation of the solution, or which act as mordants, and are useful in special cases.

We shall only give here a few of the standard solutions which are most frequently employed by experienced bacteriologists:

1. *Aniline-Gentian-Violet* (Ehrlich).

Saturated alcoholic solution of gentian violet,	5 cc.
Aniline water,	100 cc.

2. *Aniline-Methyl-Violet* (Ehrlich-Weigert).

Saturated alcoholic solution of methyl violet,	11 cc.
Absolute alcohol,	10 cc.
Aniline water,	100 cc.

Aniline water for the above solutions is prepared by shaking in a test tube one part of aniline oil with twenty parts of distilled water, and, after allowing it to stand for a short time, filtering the saturated aqueous solution through a moistened filter. If the solution is not perfectly transparent it should be filtered a second time.

3. *Carbol-Fuchsin* (Ziehl's solution).

Fuchsin,	1 gm.
Alcohol,	10 cc.

Dissolve and add 100 cc. of a five-per-cent solution of carbolic acid.

4. *Alkaline Blue Solution* (Löffler's solution).

Saturated solution of methylene blue,	30 cc.
Solution of caustic potash of 1:10,000,	100 cc.

These solutions keep better than the simple aqueous solutions, but after having been kept for a time they are likely to lose their staining power as a result of the precipitation of the aniline color.

The following special methods of staining cover-glass preparations will be found useful in certain cases:

Gram's Method.—The dried film upon a slide or cover glass is stained with an aqueous solution of methyl violet or with aniline-gentian-violet solution (No. 1); it is then placed in the iodine solution for a minute or two (iodine one part, potassic iodide two parts, water three hundred parts); then washed in alcohol, dried, and, if for permanent preservation, mounted in balsam.

METHODS OF STAINING THE TUBERCLE BACILLUS.—Numerous methods of staining the tubercle bacillus in sputum dried upon a cover glass have been proposed, but we shall only give here two or three of the most approved methods, either one of which may be relied upon for satisfactory results if carefully followed.

1. *The Ehrlich-Weigert Method.*—Place in a watch glass a little of the aniline-methyl-violet solution (No. 2); float upon the surface of this the cover glass with the dried film downward; heat over a small flame until it begins to steam, then allow it to stand for from two to five minutes; decolorize in a tray containing one part of nitric acid to three parts of water—the cover glass, held in forceps, is gently moved about in the decolorizing solution for a few seconds. It is then washed off in sixty-per-cent alcohol to remove the remaining blue color—this usually takes but a second or two—and then in water. For a contrast stain a saturated aqueous solution of vesuvin may be used, a few drops being left upon the cover glass for five minutes. The stained preparation is then washed, dried, and mounted in balsam.

2. *The Ziehl-Neelson Method.*—Float the cover glass upon the carbol-fuchsin solution (No. 3); heat gently until steam commences to rise—from three to five minutes' time will usually be sufficient; wash off in water, and decolorize in nitric or sulphuric acid, twenty-five-per-cent solution, then in sixty-per-cent alcohol for a very short time to remove remaining color from albuminous background; wash well in water and mount in Canada balsam.

3. *Friedländer's Method.*—Spread and dry the sputum upon the slide; fix by passing the slide three times through the flame of an alcohol lamp or Bunsen burner; place upon the dried film three or four drops of carbol-fuchsin (No. 3); heat gently over a flame until steam is given off; wash in a dish of distilled water; drain off excess of water, and add a few drops of the following decolorizing solution:

Acid, nitric, pure, ...	5 cc.
Alcohol (eighty per cent), ...	to 100 cc.

—usually the preparation will be decolorized in about half a minute; wash in water; add a few drops of an aqueous solution of methylene blue as a contrast stain; allow the stain to act for about five minutes, without heating; wash again in water, dry, and mount in balsam, or for a temporary mount use a drop of cedar oil.

4. *Gabbett's Method.*—This is a slight modification only of a very useful method recommended by B. Fränkel in 1884. The contrast stain is added to the decolorizing solution. After staining with

carbol-fuchsin solution (No. 3) the cover glass is placed for one or two minutes in a solution containing:

Sulphuric acid (twenty-five-per-cent solution), . .	100 cc.
Methylene blue, ...	2 gms.

Wash, dry, and mount in cedar oil or balsam.

METHODS OF STAINING SPORES.—When preparations containing the spores of bacilli are stained by any of the methods above given, these remain unstained and appear as highly refractive bodies in the interior of the rods or filaments in which they have been formed, or scattered about in the field if they have been set free. Owing to the contrast with the stained protoplasm of the rod or spore-bearing filament, they are especially well seen in recent cultures; while in older cultures the bacilli often do not stain well, or are entirely disintegrated and spores only are to be seen. The discovery was made at about the same time by Buchner (1884) and by Hueppe that spores may be stained if they are first exposed to an elevated temperature for some time. This may be accomplished by placing the slide or cover glass, upon which the spore-containing culture has been dried, in a hot-air oven at a temperature of 120° C. for an hour; or a higher temperature (180° C.) may be employed for a shorter time (fifteen minutes); or the cover glass may be passed through the flame of an alcohol lamp or Bunsen burner eight or ten times, instead of three times as is customary when the object in view is simply to coagulate the albumen and fix the film upon the cover glass. After such treatment the spores may be stained with an aqueous solution of one of the basic aniline colors—fuchsin, methyl violet, etc.—but the bacilli no longer take the stain so well.

To obtain satisfactory *double-stained preparations*, showing both spores and bacilli, a different method is employed.

The film upon the cover glass is passed through the flame three times, as heretofore directed; it is then floated upon aniline-fuchsin solution in a watch glass, and this is heated to near the boiling point for an hour—*Neisser's method.* The aniline-fuchsin solution is prepared by shaking an excess of aniline oil in a test tube with distilled water, filtering the saturated solution into a watch glass, and then adding a few drops of a saturated alcoholic solution of fuchsin. After this prolonged action of the hot staining fluid the spores of some bacilli are deeply stained, while others do not take the stain so well. The cover glass is next washed in water and then placed in a decolorizing solution containing twenty-five parts of hydrochloric acid to seventy-five parts of alcohol. This removes the stain from the bacilli, but, if not allowed to act too long, leaves the spores still stained. The preparation is next stained in a saturated aqueous solution of methylene blue; and if the operation has been successfully carried out the spores will be stained red and the protoplasm of the bacilli in which they are present will be blue.

Möller has (1891) published the following *method of staining spores:*

The cover-glass preparation, dried in the air, is passed three times through a flame or placed for two minutes in absolute alcohol; it is then placed in chloroform for two minutes and washed in water; it is now immersed in a five-per-cent solution of chromic acid for from half a minute to two minutes and again thoroughly washed in water; next a solution of carbol-fuchsin is poured upon it and it is heated over a flame until it commences to boil, for sixty seconds; the carbol-fuchsin solution is then poured off and the cover glass is immersed in a five-per-cent solution of sulphuric acid until the film is decolorized, after which it is again thoroughly washed in water. It is then placed for thirty seconds in an aqueous solution of methylene blue or of malachite green, and again washed in water, after which the preparation should be dried and mounted in balsam. As a result of this procedure the spores are stained dark red and the protoplasm of the bacilli blue or green.

Fiocca (1893) claims that better results are obtained by the following method:

About twenty cc. of a ten-per-cent ammonia solution is placed in a watch glass, and from ten to twenty drops of an alkaline solution of an aniline color is added; heat is applied until steam commences to be given off, when the cover glass is placed in the hot solution for from three to fifteen minutes. The cover glass is then quickly washed in a twenty-per-cent solution of nitric or sulphuric acid to decolorize; then it should be thoroughly washed in water, after which it may be stained with a contrast color by the use of an aqueous solution of one of the aniline dyes—preferably vesuvin, malachite green, or safranin.

METHODS OF STAINING FLAGELLA.—Koch first succeeded in demonstrating the flagella of certain bacilli and spirilla by staining them with an aqueous solution of hæmatoxylon, and dilute chromic acid as a mordant. Löffler (1889) has succeeded in demonstrating, by an improved staining method, the presence of flagella in a considerable number of species in which they had not previously been seen, although generally suspected to be present. His method is as follows:

Löffler's Method.—The following solution is used as a mordant:

No. 1.

Solution of tannin of twenty per cent,	10 cc.
Saturated (cold) solution of ferrous sulphate,	5 cc.
Aqueous or alcoholic solution of fuchsin,	1 cc.

(Or one cubic centimetre alcoholic solution of methyl violet.)

No. 2.

A one-per-cent solution of caustic soda.

No. 3.

A solution of sulphuric acid of such strength that one cubic centimetre is exactly neutralized by one cubic centimetre of the soda solution.

According to Löffler, solution No. 1 is just right for staining the flagellum of Spirillum concentricum, but for certain other bacteria it is necessary to add to this some of No. 2 or of No. 3. Thus, for the cholera spirillum from half a drop to a drop of the acid solution is added to sixteen cubic centimetres of No. 1. For the bacillus of typhoid fever one cubic centimetre of No. 2 is added to sixteen cubic centimetres of No. 1. Bacillus subtilis requires twenty-eight to thirty drops of No. 2; the bacillus of malignant œdema thirty-six to thirty-seven drops, etc.

This method has not been very successful in the hands of other bacteriologists, and improvements in the technique have been made since it was first published. Van Ermengem (1893) points out the fact that a principal condition of success is that the cover glasses shall be absolutely clean. He boils them in a mixture composed of potassium bichromate, sixty grammes; concentrated sulphuric acid, sixty grammes; water, one hundred grammes. After coming from this they are thoroughly washed in water, then in absolute alcohol, and then dried in an upright position under a bell-jar. Recent agar cultures (ten to eighteen hours) are preferred, and the suspension in water should be very much diluted so that in the cover-glass preparation the bacteria are well isolated. The cover glass, held between the fingers, is passed three times through a flame. A drop of the following solution is then placed upon it: Osmic acid two-per-cent solution, one part; solution of tannin (ten to twenty-five per cent) two parts. This is allowed to act for about five minutes at a temperature of 50° to 60° C.—or half an hour at the room temperature. After careful washing with water and alcohol the cover glass is immersed for a few seconds in a bath containing one-quarter to one-half per cent of nitrate of silver. Then without washing it is placed for a short time in the following: Gallic acid, five grammes; tannin, three grammes; fused potassium acetate, ten grammes; distilled water, three hundred and fifty grammes. It is then returned to the silver bath and kept there, with constant movement of the bath, until this commences to turn black. It is then thoroughly washed in water, dried, and mounted in balsam.

Pitfield (1895) has devised a much simpler method which, as modified by Muir, is as follows:

"Prepare the following solutions:

A.—The Mordant.

Tannic acid, ten-per-cent aqueous solution, filtered, .	10 c.c.
Corrosive sublimate, saturated aqueous solution, . .	5 c.c.
Alum, saturated aqueous solution, ...	5 c.c.
Carbol-fuchsin solution,[1]	5 c.c.

"Mix thoroughly. A precipitate forms, which must be allowed to deposit, either by centrifugalizing or simply by allowing to stand. Remove the clear fluid with a pipette and transfer to a clean bottle. The mordant keeps well for one or two weeks.

B.—The Stain.

Alum, saturated watery solution, ...	10 c.c.
Gentian violet, saturated alcoholic solution, . . .	2 c.c.

"The stain should not be more than two or three days old when used. It may be substituted in the mordant in place of the carbol fuchsin. The film having been prepared as above described, pour over it as much of the mordant as the cover glass will hold. Heat gently over a flame till steam begins to rise, allow to steam for about a minute, and then wash well in a stream of running water for about two minutes. Then dry carefully over the flame, and when thoroughly dry pour on some of the stain. Heat as before, allowing to steam for about a minute, wash well in water, dry and mount in a drop of xylol balsam" (Muir and Ritchie).

Methods of Staining Bacteria in Tissues.—The solutions recommended for staining cover-glass preparations are also used in staining bacteria in thin sections of the various organs, in which they are found in certain infectious diseases; but, in general, a longer time is required to stain sections, and it is best not to hasten the process by the use of heat. To obtain good thin sections, the material, cut in small cubes, must be very thoroughly hardened in absolute alcohol. The piece selected for cutting may be attached to a cork by the use of melted glycerin jelly, which is hardened by placing the cork and attached piece of tissue in alcohol. This answers for well-hardened pieces of liver, kidney, etc., but the hollow viscera and tissues of loose structure will require embedding in paraffin or celloidin. Any well-made sledge microtome will answer for cutting the sections, if the knife is properly sharpened. The sections should, of course, be cut under alcohol, and they can scarcely be too thin when the object is to demonstrate the presence or absence of bacteria. Very thin sections may be cut dry by embedding in paraffin having a melting point of 50° C. In this case the knife is set at a right angle to the material to be cut, and the sections are spread out upon and attached to the glass slide for staining.

One of the most useful solutions for staining tissues is Löffler's alkaline solution of methylene blue (No. 4). A freshly-prepared solution will stain sections in four or five minutes. Superfluous color is removed by immersing the sections in diluted alcohol or in a one-half-per-cent solution of acetic acid for a few seconds. The sections are dehydrated in absolute alcohol, cleared up with oil of cedar, and mounted in a drop of cedar oil for examination, or in balsam if they are to be preserved.

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[1] Basic fuchsin, 1 part; absolute alcohol, 10 parts; solution of carbolic acid (1:20), 100 parts.

Gram's method may be used as directed for cover-glass preparations, the sections being first stained in aniline-gentian-violet solution (No. 1), then washed in water, or in aniline water as recently (1892) recommended by Botkin, then decolorized in the iodine solution (see page 29). The sections when decolorized are again washed in water, dehydrated in absolute alcohol, cleared in cedar oil, and mounted in balsam.

Weigert's Method.—This is a modification of Gram's method in which the sections are dehydrated by the use of aniline oil. The stained section, after having been washed, is transferred to a clean glass slide, the excess of water is removed by the use of filtering paper, and the iodine solution is placed upon it in sufficient quantity to cover the entire section. When sufficiently decolorized this is removed in the same way. The section is then dehydrated by placing a few drops of aniline oil upon it, removing this with filtering paper, and repeating the operation once or twice. The aniline oil must then be completely removed by the use of xylol, after which the section is mounted in balsam.

Kühne's Method.—The object of this method is to prevent the removal of the color from stained bacteria in sections during the treatment which such sections usually receive before they are ready for mounting—*i.e.*, during the washing and dehydrating processes usually employed. For staining, Kühne prefers a methylene-blue solution prepared as follows: Methylene blue, 1.5 parts; absolute alcohol, ten parts; triturate in a watch glass and add gradually one hundred parts of a solution of carbolic acid containing five parts in one hundred of water. The section is placed in this solution for about half an hour, then washed in water and decolorized in a weak solution of hydrochloric acid—ten drops to five hundred grammes of water. This part of the operation must be conducted very carefully, and usually thin sections will only require to be dipped in the acid solution for an instant, after which they must be at once immersed in a solution of lithium—eight drops of a saturated solution of carbonate of lithium in ten grammes of water. They are then allowed to remain in a bath of distilled water for a few minutes, after which they are dipped into absolute alcohol, which Kühne colors by the addition of methylene blue. The sections are then placed in aniline oil which contains a little methylene blue in solution, where they are dehydrated without the color being extracted from the stained bacteria present. The aniline-oil blue solution is prepared by adding an excess of dry methylene blue to a small quantity of clarified aniline oil. The undissolved pigment settles to the bottom, and a few drops of the colored solution are added to a little aniline oil in a watch glass to make the colored dehydrating bath. The section is next washed out in pure aniline oil—not colored—after which every trace of aniline oil is to be removed by the use of xylol. The section is cleared up in turpentine and mounted in balsam.

Ziehl-Neelson Method, for the tubercle bacillus in tissues.—Leave the sections for fifteen minutes in carbol-fuchsin solution (No. 3); decolorize in sulphuric or nitric acid, twenty-five-per-cent solution; wash in sixty-per-cent alcohol; place in a saturated aqueous solution of methylene blue for contrast stain; wash, dehydrate, and mount in balsam.

The following method of staining sections for the purpose of demonstrating bacteria present in the tissues is recommended by Pregl (1891) as a *substitute for the method of Kühne.* The results are said to be excellent, and it is much simpler and more expeditious.

The sections are made from tissues embedded in paraffin, and are attached to clean glass slides with albumen-glycerin. Or they may be attached to a cover glass by the following method when not embedded in paraffin: The sections, completely dehydrated, are taken out of absolute alcohol on a thin glass cover, upon which they are extended; a piece of filter paper is applied to the side of the cover glass to absorb the alcohol, and before the section is completely dry a drop of aceton-celloidin solution is placed upon it by means of a glass rod. The cover glass is now moved about in the air to promote rapid evaporation of the alcohol, and is then placed in water. The section now remains attached to the cover glass during subsequent manipulations. The *aceton-celloidin solution* referred to is prepared by adding celloidin in small, dry pieces to aceton until a concentrated solution is obtained. A large drop of this added to five cubic centimetres of absolute alcohol makes a suitable solution for use. This must be kept in a glass-stoppered bottle, and will require to be frequently renewed, as it is not suitable for use after having absorbed moisture from the air. The aceton as obtained from dealers contains considerable water and must be dehydrated by adding to it red-hot sulphate of copper.

The sections, attached to a slide or cover glass by one of the methods mentioned, are stained with Kühne's carbol-methylene-blue solution, which is dropped upon them from a pipette. Usually they will be sufficiently stained at the end of half a minute to a minute, but in some cases a longer time and the application of heat will be desirable. They are then washed in water and immediately placed in fifty-per-cent alcohol, where they remain until the sections have a pale-blue color with a greenish tinge. They are now completely dehydrated in absolute alcohol and subsequently cleared up in xylol.

STAINING SECTIONS OF GELATIN STICK CULTURES.—Fischl, Weigert, and Neisser have given an account of methods for staining stick cultures in gelatin of non-liquefying bacteria. The object of this is to show the mode of growth and the association of individual cells in undisturbed cultures. Neisser gives the following directions: The gelatin cultures are inoculated, by several punctures, with the microörganism to be studied. When the development is deemed sufficient the cylinder of gelatin is removed from the test tube by gently warming its walls. It is then placed for several days—one to eight, according to its size and thickness—in a one-per-cent solution of bichromate of potassium. While in this solution it must be exposed to the light, which causes a change in the gelatin, rendering it insoluble. The gelatin cylinder is thoroughly washed and then hardened in alcohol, first of seventy per cent. and then of ninety-six per cent. It is then cut into suitable pieces, and these are attached to a cork in the usual manner and placed for twenty-four hours in absolute alcohol. Thin sections may now be made with a microtome, and these are attached to a glass slide and stained by Gram's or Weigert's method or by the use of Löffler's solution (No. 4). The decolorization should be effected by the use of alcohol and not with an acid solution. When Gram's method is used decolorize by the alternate use of alcohol and oil of cloves. Clear the preparation with oil of bergamot.

V.

CULTURE MEDIA.

To obtain a satisfactory knowledge of the biological characters of the different species of bacteria, it is necessary to isolate them in "pure cultures" and to study their growth in various culture media. By a pure culture we mean a cultivation containing a single species only; and to be absolutely sure that we have a pure culture it is desirable that all of the bacteria in a culture shall be the progeny of a single cell. The methods of obtaining pure cultures will be given later. At present we propose to give an account of the various culture media commonly employed by bacteriologists, and the methods of preparing them for use.

By a natural culture medium we mean one which, as obtained in nature, contains the necessary pabulum for the development of one or more species of bacteria. An artificial culture medium is one which is prepared artificially by adding nutritive material to water. A sterile medium is one which does not contain any living microörganisms. We may obtain natural media in a sterile condition, but artificial media require sterilization, as they are infallibly contaminated with living "germs" from the atmosphere during the process of preparing them. Sterilization is usually effected by heat. Forceps, glass tubes, etc., may be sterilized by passing them through the flame of an alcohol lamp or Bunsen burner.

Natural Culture Media.—The most important natural culture medium is *blood serum*, which may be obtained from one of the lower animals—preferably from oxen or calves. This is to be collected in a sterilized jar, with every precaution to insure cleanliness, at the moment of slaughtering the animal. Or the blood of a calf, sheep, or dog may be collected at the laboratory by a carefully conducted operation, in which the femoral or carotid artery is connected with a sterilized glass tube leading into a sterilized receptacle, such as a Woulf's bottle, into one neck of which a cotton plug has been placed to permit the air to escape as the bottle fills with blood through a tube which is secured in the other neck. When blood is passed directly from an artery into a sterilized receptacle the serum will not subsequently require sterilization. The writer is in the habit of collecting it in this way, and, after the serum has separated, of drawing it off in little flasks having a long neck, as shown in Fig. 14. The neck of the flask, previously sterilized by heat, is slipped into the Woulf's bottle beside the cotton plug, the bulb (*a*) having been previously gently heated to expand the contained air. As the heated air cools a partial vacuum is formed and the clear serum mounts into the little flask. One after another is filled in this way, and each one is hermetically sealed in the flame of a lamp as soon as it is withdrawn. The sterile blood serum may be preserved indefinitely in this way, and may be used as a liquid culture medium in the little flask, or it may be transferred to a test tube and solidified by heat whenever a solid blood-serum medium is required. The advantage of preserving blood serum and other liquid media in these little flasks is in the fact that they may be preserved indefinitely without becoming contaminated or drying up, and that they are easily transported, while a liquid medium in a test tube must be kept upright. The contents of one of these flasks are readily

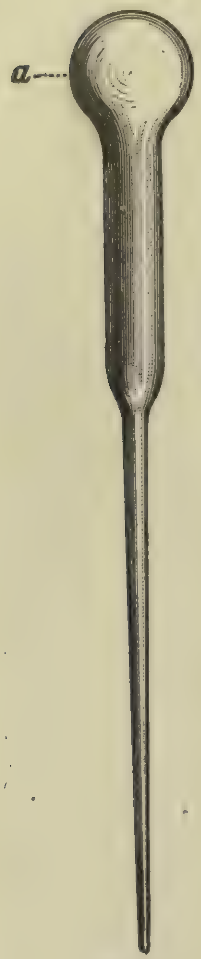

FIG. 14.

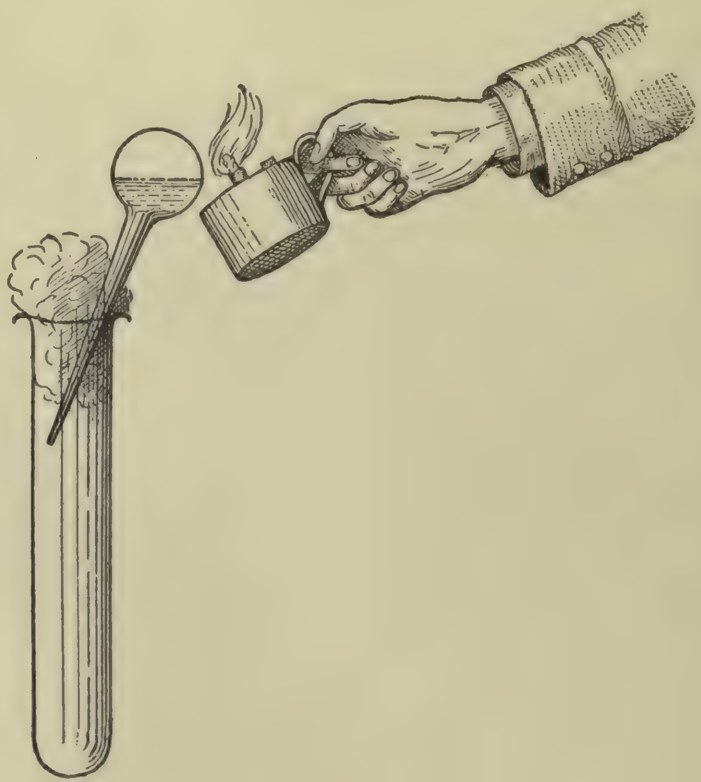

FIG. 15.

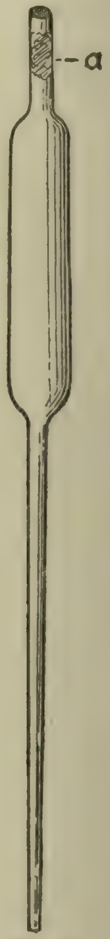

FIG. 16.

transferred to a test tube by breaking off the sealed extremity with sterile forceps and slipping it past the cotton plug, which must be partly withdrawn for the purpose. Upon applying gentle heat to the bulb its contents are forced out into the test tube (Fig. 15). Blood serum which is collected without these special precautions will require sterilization by heat, for which directions will be given later.

To obtain the clear serum from blood collected as above directed, the jars containing it are set aside in a cool place in order that a firm clot may form, care being taken not to shake them. After the clot has formed they may be transported to the laboratory, where they are placed in an ice box or in a cool cellar for from twenty-four to forty-eight hours. By this time the serum has separated from the clot, and it may be transferred to sterilized test tubes by means of a suction pipette (Fig. 16), or may be distributed in little flasks as above directed

Milk is largely used as a culture medium, and is especially useful in studying the biological characters of various microörganisms, as shown by their causing coagulation of the casein, or otherwise; or an acid or alkaline reaction of the liquid; or peptonization of the precipitated casein, etc. In the udder of healthy cows milk is quite sterile, and by proper precautions it may be drawn into sterilized flasks without any contamination and kept indefinitely without undergoing coagulation or any other change. But in practice it is easier to sterilize it in test tubes or small flasks by the use of heat than to obtain it in a sterile condition from the udder of the cow.

Urine has been used to some extent as a culture medium, and many bacteria multiply in it abundantly, although, on account of its acid reaction, other species fail to grow in it. As contained in the healthy bladder it is sterile, but the mucous membrane of the meatus urinarius always contains numerous bacteria upon its surface, and some of these are sure to be carried away with the current when urine is passed.

A culture fluid which the writer has found extremely useful, in tropical countries where it is to be obtained, is the transparent fluid contained in the interior of unripe cocoanuts—called *agua coco* by the Spaniards. In countries where the cocoanut is indigenous this cocoanut water is largely used as a refreshing drink. It contains about four per cent of glucose in solution, together with some vegetable albumen and salts. Some microörganisms multiply in it without appropriating the glucose, while others split this up, producing an abundant evolution of carbon dioxide and giving to the fluid a very acid reaction. The following are the results of an analysis made for me by Dr. L. L. Van Slyke in the chemical laboratory of Johns Hopkins University: The weight of the fluid obtained from six nuts averaged 339.1 grammes. The specific gravity averaged 1.02285. The amount of water averaged 95 per cent; the amount of inorganic ash, 0.618 per cent; the amount of glucose, 3.97 per cent; the amount of fat, 0.119 per cent; the amount of albuminoids, 0.133 per cent.

As this fluid is contained in a germ-proof receptacle, no sterilization is required when it is drawn off with proper precautions in the little flasks heretofore described.

Hydrocele fluid has been used as a culture medium, and many bacteria multiply in it abundantly.

Other natural culture media are found in animal and vegetable substances, which are used, either cooked or raw, as solid substrata upon which bacteria may be cultivated. One of the most useful of these is the *potato*, which is a favorable medium for the development of numerous species, and upon which (cooked) many of them present characters of growth which are so distinctive as to aid greatly in the differentiation of species.

Other *tubers, roots*, or *fruits* may also be used as solid media, or their juices extracted and employed as liquid media. Cooked fish and meats of various kinds are also suitable media for certain species—*e.g.*, the phosphorescent bacteria grow very well upon the surface of boiled fish, and in a dark room give off a bright, phosphorescent light.

Eggs, sterilized by boiling, have been used by some bacteriologists, especially for the cultivation of anaërobic species.

ARTIFICIAL CULTURE MEDIA.—A great variety of liquid media have been employed by bacteriologists, the most useful of which are infusions of beef or mutton, with the addition of a little peptone. But Pasteur has shown that some species of bacteria will grow in a medium which does not contain any albuminous material, nitrogen being obtained from salts containing ammonia.

Pasteur's solution, which is rarely used at present, contains: Distilled water, one hundred parts; cane sugar, ten parts; tartrate of ammonia, one part, with the addition of the ashes from one gramme of yeast.

Cohn modified this by leaving out the cane sugar, which favors the development of moulds. These fluids are not, however, intended for general use in the cultivation of bacteria, but to demonstrate certain facts relating to their physiology.

Infusions of meat, or "flesh water," are made by chopping fine lean beef or mutton (one pound) and covering it with water (one litre). This is placed in an ice chest for twenty-four hours, and the aqueous extract is then obtained by filtration through muslin by pressure. This extract is cooked, filtered, and carefully neutralized by the addition of a solution of carbonate of sodium, which is added drop by drop. Usually we add to this one-half per cent of chloride of sodium. The addition of ten grammes of peptone to a litre of this meat infusion constitutes the *flesh-peptone solution* which is largely used in the preparation of solid culture media, to be described hereafter.

The addition of five per cent of glycerin to the above infusion makes a useful liquid medium for the cultivation of the tubercle bacillus (Roux and Nocard). The liquid should be again neutralized after adding the glycerin, which commonly has an acid reaction.

Dunham's Peptone Solution.—This is used principally for determining whether bacteria under investigation are capable of producing indol. One part of *pure* dried peptone is added to 100 parts of distilled water, and to this is added one-half per cent of sodium chloride. The addition of rosalic acid to this solution affords a means of determining whether bacteria cultivated in it produce an acid or an alkaline reaction of the medium. The pale rose color imparted to the peptone solution by the addition of rosalic acid becomes more intense when the solution becomes alkaline, and it fades out entirely when it becomes acid. To obtain this reaction add 2 parts of the following solution to 100 parts of Dunham's peptone solution: rosalic acid (corralline), 2 parts; alcohol (eighty per cent), 100 parts.

Bouillon is made by cooking the chopped meat—one pound in a litre of water—for about half an hour in a large glass flask or an enamelled iron kettle. The filtered bouillon is then carefully neutralized with sodium carbonate, and again boiled for an hour to precipitate all coagulable albuminoids. It is again filtered and distributed in test tubes or small flasks, in which it is subsequently sterilized. For certain pathogenic bacteria a bouillon made from the flesh of a fowl or of a rabbit is preferable to beef bouillon.

Flesh infusion may also be made from one of the standard beef extracts, such as Liebig's (five grammes to a litre of water).

Various *vegetable infusions* may also be used as culture media, such as yeast water, potato water, infusion of hay, of barley, or of wheat, of dried fruits, beer wort, etc.

SOLID CULTURE MEDIA.—The introduction of solid culture media, and especially the use of gelatin and agar-agar, as first recommended by Koch (1881), for the isolation and differentiation of species, was a most important advance in bacteriological technology. We are concerned here only with the composition and preparation of these media.

Flesh-Peptone-Gelatin.—This is made by adding ten per cent of the best French gelatin to the flesh-peptone solution above described. This is the standard gelatin medium, but more or less gelatin may be added to serve a special purpose. Thus, in Havana during the summer months the writer used a medium containing twenty per cent of gelatin, because when but ten per cent was used the gelatin was liquefied by the normal temperature of the atmosphere. Ten-per-cent gelatin, of good quality and carefully prepared, will stand a temperature of 20° to 22° C. (68° to 71.6° F.) without melting. When twenty per cent of gelatin is used the melting point is about 8° C. higher. It must be remembered that exposure to a boiling temperature reduces the melting point of gelatin. It is therefore desirable to accomplish the operations of cooking and sterilizing in as short a time as is practicable. The French gelatin used comes in thin sheets; this is broken up and added to the flesh-peptone solution.

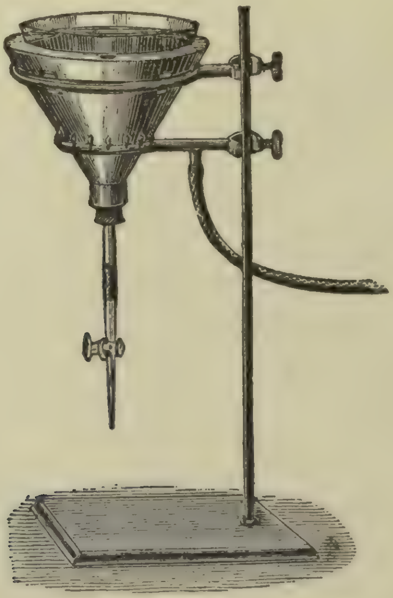

FIG. 17.

Usually we prepare a litre of nutrient gelatin at one time, and for this quantity one hundred grammes of gelatin will be required for the standard preparation (ten per cent). It is well to allow it to soak for a time in the liquid before applying heat for the purpose of dissolving it. Then apply gentle heat until it is completely dissolved. The gelatin of commerce usually has an acid reaction, and it will be necessary to carefully neutralize the medium after it has been added. A slightly alkaline reaction is usually no disadvantage, but certain pathogenic bacteria will not grow when there is a trace of acid present. The next step consists in *clarifying* the nutrient medium. It is allowed to cool to about 50° C., and an egg, previously broken into one hundred grammes of water, is gradually added while stirring the liquid with a glass rod. A whole egg is used for a litre of the solution. Heat is again applied and the solution is kept at the boiling point for about ten minutes, during which time the egg albumen is precipitated and carries down with it all insoluble particles, which without this clarifying process would have interfered with the transparency of the medium, even when carefully filtered. The hot solution is then *filtered*. A hot-water funnel (Fig. 17) is usually employed, as the gelatin solution does not pass through filtering paper very rapidly, and when cooled to near the point of solidifying ceases to pass.

The advantages of the gelatin medium are that it is perfectly transparent, that it is easily melted for making "plates," and that many bacteria exhibit in it special characters of growth by which they may be differentiated from others which resemble them in form. The principal disadvantage is the low melting point, which prevents us from making use of this medium for cultivating bacteria in an incubating oven at a higher temperature than about 22° C. for ten-per-cent gelatin.

This disadvantage is overcome by using *agar-agar* instead of gelatin. This is prepared in Japan and other Eastern countries from certain species of gelatinous algæ. It comes to us in the form of bundles of dried strips, which form a stiff jelly when dissolved in water in the proportion of one to two per cent. This jelly remains solid at a temperature of 40° C. and above. It was first employed by Hesse, one of Koch's collaborators in the office of the imperial board of health of Berlin. Koch, who was in search of a transparent jelly which would stand the temperature required for the cultivation of certain pathogenic bacteria (37° to 38° C.), quickly recognized its value and introduced it into general use.

The *agar-agar jelly* is more difficult to filter than the gelatin medium, and some skill is required in order to obtain a transparent solution. It will bear long boiling without losing its quality of forming a stiff jelly. From ten to twenty grammes are added to a litre of flesh infusion, or we may make a *peptonized agar* in accordance with the following formula which is given by Salomonson: Add to one litre of distilled water five grammes Liebig's extract, thirty grammes peptone, five grammes cane sugar, fifteen grammes agar. Cook for an hour, render slightly alkaline, and cool to below 60° C. Clarify and cook again for an hour or more.

Glycerin-agar is made by adding five per cent of glycerin to the peptonized agar made by the above formula or by the use of the flesh-peptone infusion. This is a very favorable medium for the cultivation of the tubercle bacillus—first used by Roux and Nocard.

Agar-gelatin, a medium which has recently come into favor and is said to be very useful, as it resembles gelatin in transparency and has a considerably higher melting point than ten-per-cent gelatin, is made by adding fifty grammes of gelatin and 7.5 grammes of agar to a litre of flesh-peptone solution. Care should be taken not to cook this longer than is necessary.

In making all of these agar culture media the main difficulties encountered result from the difficulty of dissolving the agar and the slowness with which the solution passes through filtering paper. These difficulties are best met as follows: Break up the sticks of agar into small fragments and allow them to soak in cold water for twenty-four hours. Pour off the water and add the flesh-peptone solution. Boil for several hours until the agar is completely dissolved. Neutralize by adding gradually a solution of carbonate of soda (or render slightly alkaline). Filter.

The last operation is the most troublesome, and various plans have been proposed to avoid the tedious filtration through filtering paper in a hot-water filter. A method which gives satisfactory results is to place the filter containing the hot agar solution, and the flask which is to receive the filtrate, in a steam sterilizing apparatus, where it is left in an atmosphere of streaming steam until the filtration is completed. Or the solution may be put in a tall jar and left in the steam sterilizer for several hours until it is clear as a result of sedimentation. The clear solution is then obtained by decantation. Or by conducting the operation in a tall cylindrical vessel, and allowing sedimentation to occur in the steam sterilizer and the agar subsequently to solidify by cooling, the cylinder of jelly may be removed from the jar and the part containing the sediment can be cut away. The transparent portion is then melted again and distributed in test tubes for use.

In the present volume we frequently refer to the nutrient medium made by adding one to two per cent of agar-agar to the standard flesh-peptone solution as "nutrient agar" or simply as "agar."

The following method of filtering agar has (1890) been proposed by Karlinsky. It is a modification of the method previously described by Jakobi and depends upon the use of pressure.

In Fig. 18, *a* is a cylindrical vessel of tin, which is closed above by a perforated rubber cork, through which is passed a glass tube, *b*. This is enclosed in a larger tin cylinder, *c*, which contains water, which may be kept hot by placing an alcohol lamp under the projecting arm *d*. The central cylinder has a tube, *e*, passing through the bottom of the hot-water cylinder, and which is provided with a stopcock for drawing off the filtered solution. Before pouring the hot agar solution into the cylinder *a*, a cotton filter about ten centimetres thick is placed at the bottom of this cylinder and hot water is poured upon it while the stopcock of the outlet tube is open. This washes out the cotton and prepares the filter for the agar solution. The apparatus is supported upon a tripod, not shown in the figure. Filtration is said to occur rapidly when the air in the central cylinder is compressed by means of the hand bellows attached to the tube *b*.

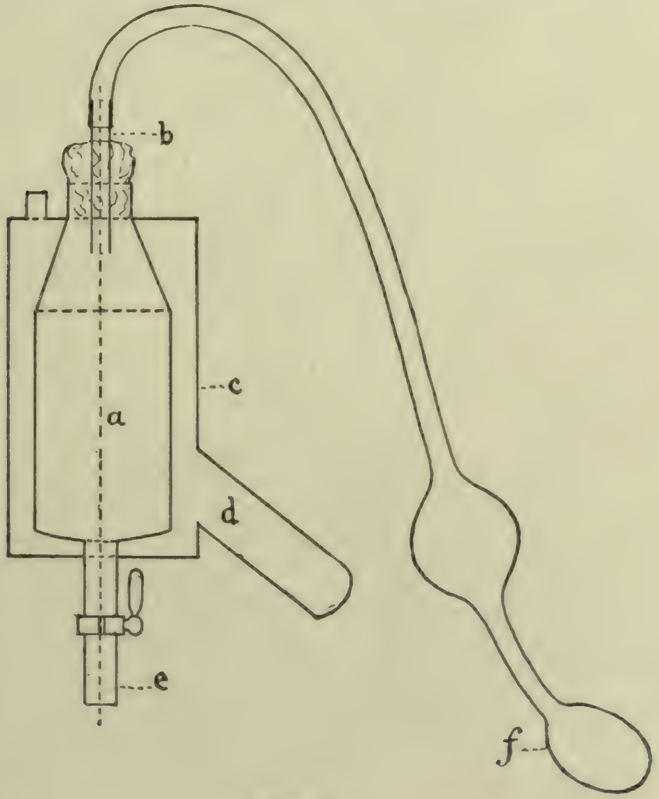

FIG. 18.

Schultz' Rapid Method of Preparing Nutrient Agar-Agar.—Place one thousand five hundred cubic centimetres of water in an enamelled iron pot; add eighteen grammes of agar-agar, broken in small pieces, and place upon a gas stove; boil for half an hour; add while boiling two grammes of Liebig's extract of beef; remove from fire and cool to 60° C.; then add ten grammes of dry peptone, five grammes of sodium chloride, and the contents of one egg beaten up in a sufficient quantity of water to supply that lost by evaporation; neutralize the mixture by the addition of dilute hydrochloric acid; boil again for five or ten minutes; filter through white filter paper. If the filtrate is not entirely clear add to it the albumen of a second egg and boil until this is coagulated; then filter again. *Always moisten the filter with water before filtering solutions containing gelatin or agar-agar.* When the process is completed the amount of filtered culture medium should be about one thousand cubic centimetres.

According to Abbott the filtration of agar-agar does not require the use of a hot-water funnel or any other device for maintaining the temperature of the mass. He gives the following directions for its preparation:

> "Prepare the bouillon in the usual way. Agar-agar reacts neutral or very slightly alkaline, so that the bouillon may be neutralized before the agar-agar is added. Then add finely chopped or powdered agar-agar in the proportion of one to 1.5 per cent. Place the mixture in a porcelain-lined iron vessel, and on one side of the vessel make a mark at the height at which the level of the fluid stands. If a litre of medium is being made, add about two hundred and fifty to three hundred cubic centimetres more water, and allow the mass to boil slowly, occasionally stirring, over a free flame, from one and a half to two hours; or until the excess of water—*i.e.*, the two hundred and fifty or three hundred cubic centimetres that were added—has evaporated. Care must be taken that the liquid does not boil over the sides of the vessel. From time to time observe if the fluid has fallen below its original level; if it has, add water until its volume of one litre is restored. At the end of the time given remove the flame and place the vessel containing the mixture in a large dish of cold water; stir the agar-agar continuously until it has cooled to about 68° to 70° C., and then add the white of one egg which has been beaten up in about fifty cubic centimetres of water; or the ordinary dried albumen of commerce may be dissolved in cold water in the proportion of about ten per cent and used—the results are equally as good as when eggs are employed. Mix this carefully throughout the agar-agar and allow the mass to boil slowly for about another half-hour, observing all the while the level of the fluid, which should not fall below the litre mark. It is necessary to reduce the temperature of the mass to the point given, 68° to 70° C.; otherwise the coagulation of the albumen will occur suddenly in lumps and masses as soon as it is added, and its clearing action will not be uniform. The process of clarification with the egg is purely mechanical; the fine particles, which would otherwise pass through the pores of the filter, being taken up by the albumen as it coagulates and being retained in the coagula. At the end of one-half hour the boiling mass may be easily and quickly filtered through a heavy-folded paper filter at the room temperature."[1]

For special purposes various substances are added to the above-described solid and liquid media. A favorable addition for the growth of a considerable number of bacteria is from one to three per cent of *glucose*. The phosphorescent bacteria grow best in a medium containing two to three per cent of *sodium chloride*. The addition of three to four per cent of *potassium nitrate* is made in conducting experiments designed to test the reducing power of certain bacteria, by which this salt is decomposed with the production of nitrites. *Acids* are also added in various proportion to test the ability of bacteria under investigation to grow in an acid medium. From 1:2,000 to 1:500 of hydrochloric acid may be used for this purpose. The addition of *litmus* to milk or other culture media is frequently resorted to for the purpose of ascertaining whether acids or alkalies are developed during the growth of bacteria under investigation. The addition of *aniline colors* which are variously changed by the products of growth of certain species has also been resorted to in the differentiation of species. Various disinfecting agents, such as *carbolic acid*, etc., have also been used for the same purpose, and it has been shown by experiment that some bacteria will grow in a medium containing such agents in a proportion which would entirely restrain the development of others.

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[1] Abbott's "Principles of Bacteriology" Fifth edition. pp. 100 and 101.

The *soluble silicates* which form a jelly-like mass have been proposed as a culture medium for certain bacteria which do not grow in the usual media. Kühne (1890), Winogradsky (1891), and Sleskin (1891) have made experiments which indicate that this medium has considerable value.

Winogradsky uses in the preparation of his silicate jelly the following salts :

Ammonium sulphate,	0.4 gramme.
Magnesium sulphate,	0.05 "
Potassium phosphate,	0.1 "
Calcium chloride,	a trace.
Sodium carbonate,	0.6 to 0.9 gramme.
Distilled water,	100 grammes.

To this he adds a solution of silicic acid. According to Kühne, a solution containing 3.4 per cent of silicic acid and having a specific gravity of 1.02 may be preserved in a liquid condition. To this the salts are added in greater or less amount, according to the consistence desired.

Sleskin states that a suitable jelly is formed by the addition of 1.15 to 1.45 per cent of the salts, and recommends that concentrated, sterilized solutions be added to the acid. He dissolves separately, in as little water as possible, the sulphates, the potassium phosphate and sodium carbonate, and the calcium chloride.

The use of a culture medium containing an extract from the jequirity seeds has been recommended by Kaufmann (1891), who has found, by experimenting upon various bacteria, that such a medium is useful in differentiating species.

The *jequirity solution*, which may be used as a liquid medium or may be employed in the preparation of nutrient gelatin or agar, is prepared as follows : Ten grammes of jequirity seeds are bruised in a mortar and the shells removed ; they are then placed in one hundred cubic centimetres of water and cooked for two hours in the steam sterilizer ; after allowing the infusion to cool it is filtered. The filtered liquid has a pale-yellow color and a neutral or slightly alkaline reaction. Certain bacteria grow in this solution without producing any change in its color ; others, which produce an acid reaction, cause it to be decolorized ; others, which produce an alkaline reaction of the medium, change the color to green.

Lactose Litmus-Agar.—This medium is useful for the detection of the typhoid bacillus in mixed cultures, *e.g.*, in fæces. It is made by adding to nutrient agar-agar, having a slightly alkaline reaction, two or three per cent of lactose and enough tincture of litmus to give the culture medium a pale blue color. Colonies of bacteria growing in this medium which cause a fermentation of the lactose, with formation of acid, have a pale pink color, extending to the surrounding medium. Colonies which do not give rise to acid production are pale blue. Thus, colonies of the colon bacillus would be red and colonies of the typhoid bacillus blue.

Blood-serum Mixture of Löffler.—This consists of three parts blood serum and one part of neutral meat infusion, containing one per cent of glucose. It is sterilized and solidified as directed for blood serum, but a higher temperature is required for coagulation of the mixture than for plain blood serum.

Cooked Potato.—Schröter first used cooked potato as a culture medium for certain chromogenic bacteria (1872), and Koch subsequently called attention to the great value of potato cultures for differentiating species. His plan of preparing potatoes is as follows: Sound potatoes are chosen in which the epidermis is intact. These are thoroughly washed and scrubbed with a brush to remove all dirt. The "eyes" and any bruised or discolored spots are removed with a sharp-pointed knife. They are again thoroughly washed in water, and are then placed for an hour in a bath containing mercuric chloride in the proportion of 1:500, to thoroughly disinfect the surface. They are then placed in a steam sterilizer for about three-quarters of an hour, and after an interval of twenty-four hours are again steamed for fifteen minutes. It is well to wrap each potato in tissue paper before placing it in the bichloride bath, and to leave it in this protecting envelope until it is placed in the glass dish in which it is preserved from contamination by atmospheric germs after being inoculated with some particular microörganism. Just before such inoculation the potato is cut in halves with a sterilized (by heat) table knife. The bacteria to be cultivated are placed upon the cut surface and the potato is preserved in a glass dish (Fig. 20).

A more convenient method, and one which secures the potato more effectually from atmospheric organisms, is to cut a cylinder, about an inch in diameter, from a sound potato, by means of a tin instrument resembling a cork borer or apple corer. This cylinder is cut obliquely into two pieces having the form shown in Fig. 22, and each piece is placed in a large test tube having a cotton air filter, in which it is sterilized. This method, first employed by Bolton, has been slightly modified by Roux, who recommends that a receptacle for catching the water which separates during the sterilizing process be formed by making a constriction around the test tube an inch above its lower extremity. This is done by the use of a blowpipe.

The cylinder of potato rests upon the constricted portion of the tube, as shown in Fig. 21.

Sometimes a *potato paste* is employed. The potatoes are boiled for an hour and the skins removed, after which they are mashed with a little sterilized water, placed in suitable plates, and sterilized by exposure for half an hour on three successive days in the steam sterilizer. *Bread paste* may be made in the same way, and is a very favorable medium for the growth of certain bacteria and also for the common moulds.

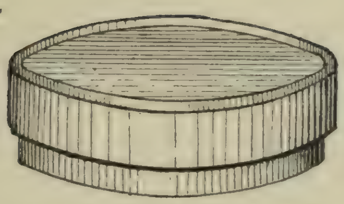

FIG. 20.

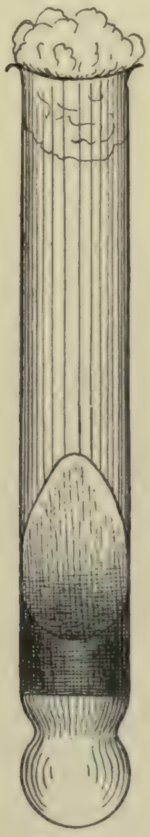

FIG. 21.

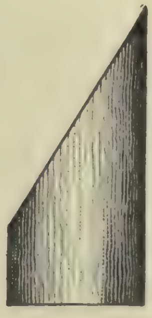

FIG. 22.

Neutralization of Culture Media. — For ordinary purposes neutralization of acid culture media is accomplished by the use of a saturated solution of sodium carbonate, the reaction being tested with strips of blue and red litmus paper. But for certain investigations it is essential that a more sensitive and reliable indicator should be used, and that an exact method of titration be employed. Schultz (1891) recommends the use of phenolphthalein as an indicator and titration with a solution of caustic soda (four-per-cent stock solution, to be diluted to 0.4 per cent for use). One drop of phenolphthalein solution, containing one gramme to three hundred cubic centimetres of alcohol, should be added to one cubic centimetre of bouillon. The beginning of an alkaline reaction is indicated by the appearance of a faint rose color. Fuller (1895), who has made a careful investigation of this subject, recommends a modification of the method of Schultz. He gives the following directions in his paper published in the *Journal of the American Public Health Association* (Vol. XX., p. 386):

> This indicator is prepared by dissolving five grammes of commercial phenolphthalein in one litre of fifty-per-cent alcohol. It is not feasible to use this indicator on strips of paper as the alcohol quickly evaporates, leaving the powder. The colorless liquid, however, may be added in small quantities to solutions of an acid or neutral nature without any change of color, but alkalies quickly change it to a purple red. This change from no color to one of purple red makes the indicator a very satisfactory one, owing to the ease with which the eye detects the so-called end-point.
>
> For the determination of the degree of reaction of nutrient media it is the custom to put five cubic centimetres (practically five drachms) of the solution into a six-inch porcelain evaporating dish, together with forty-five cubic centimetres of distilled water. This liquid is boiled for three minutes, after which is added one cubic centimetre of the phenolphthalein solution. While the solution is still hot it is quickly titrated against a twentieth normal solution of caustic alkali.
>
> As already mentioned, Dahmen stated that this indicator is useless, owing to the presence of carbonates, and of ammonia and its salts. On general grounds the point that this indicator is inaccurate under such conditions is well taken, but so far as its application to nutrient media is concerned it has been found that by proper precautions these objections may be overcome.
>
> With regard to the amount of free and combined ammonia present in ordinary nutrient media at the times when their reaction is determined, it has been found that it does not exceed 0.003 per cent. Experiments show that this quantity is less than one-tenth of that necessary to interfere with the accuracy of the method. It may be added that the reason why no ammonia is produced by the addition of alkali to the nitrogenous bodies is that at no time during the preparation is there an appreciable excess of free alkali present.
>
> The chief point by which the presence of carbon dioxide is obviated is by the use of caustic soda instead of sodium carbonate for neutralization, as referred to beyond. It has been learned by actual experiment that the carbon dioxide is practically all removed by heat during the preparation of the media and at the time of boiling just prior to the titration. In order to prevent atmospheric absorption of this gas the titration should be made quickly and in a hot solution.
>
> The remaining precaution concerns the solution against which the nutrient media are titrated. All of the usual media react acid to phenolphthalein; hence the solution in question must be alkaline. Caustic soda serves the purpose well, and the strength may be conveniently one-twentieth normal, equal to two grammes per litre. For the sake of prevention of interference from carbon dioxide in the standard solution as it meets the indicator, it is well to add a small quantity of calcium hydrate in order to precipitate this gas as calcium carbonate and allow it to settle out in the reagent bottle. After this solution has been accurately prepared, care is necessary in order to keep it of uniform strength and free from carbon dioxide. This is best done by placing the stock solution bottle on a shelf from which the liquid may be delivered into the burette by means of a siphon that is connected tightly with the top of the burette. In the tightly fitting stopper of the bottle

are three perforations: one through which the siphon passes, and another for a U tube filled with concentrated caustic soda in order to absorb the carbon dioxide from all the air which enters the bottle. The third perforation is for a by-pass which connects with the siphon just above the top of the burette and below the cock by which the flow from the bottle is regulated. The object of this is to provide for the entrance into the burette, as the solution is allowed to run from it, of air that has passed through the U tube and has had its carbon dioxide removed.

When the manipulation is carried out uniformly in the manner thus described, and with the constant employment of an end-point which has the same intensity of color, very satisfactory and closely agreeing results may be obtained by this method.

VI.

STERILIZATION OF CULTURE MEDIA.

A MOST important part of bacteriological technology consists in the sterilization of the various culture media employed. A sterile medium is essential for maintaining a pure culture, and we can only obtain an exact knowledge of the biological characters of a species by studying its growth in various media, its physiological reactions, its pathogenic power, etc., independently of all other microörganisms—*i.e.*, in pure cultures.

We may sterilize a culture medium either by heat or by filtration through a substance which does not permit bacteria to pass. The last-mentioned method is useful for certain special purposes; but, in general, sterilization of culture media, and of the vessels in which they are preserved, is effected by heat.

The scientific use of heat as an agent for sterilizing our culture media depends upon a knowledge of the thermal death-point of the various microörganisms which are liable to be present in them, and upon various facts relating to the manner in which heat is applied. All this has been determined by experiment, and before giving practical directions for sterilization it will be well to consider the experimental data upon which our methods are based.

As a rule, bacteria which do not form spores are killed at a comparatively low temperature. Thus, in a series of experiments made by the writer upon the thermal death-point of various pathogenic organisms, the pus cocci were found to be the most resistant, and all of these were killed by exposure for ten minutes to a temperature of 62° C. (143.6° F.). There are several species of bacteria known, however, which not only are not killed by this temperature, but are able to grow and multiply at a temperature of 65° to 70° C. (Miquel, Van Tieghem, Globig). But it is safe to say that exposure to a boiling temperature for a minute or two will infallibly destroy all microörganisms in the absence of spores, *when they are in a moist condition or moist heat is used—i.e.*, when they are directly exposed to the action of boiling water or of steam. The power of dry heat to destroy microörganisms in a desiccated condition is a different matter and will require special consideration.

The spores of bacilli have a much greater resisting power, and the vitality of some of these reproductive bodies, from known species, is not destroyed by a boiling temperature maintained for several hours. Thus Globig found that the spores of a certain bacillus from the soil—his "red potato bacillus"—required six hours' exposure to streaming steam in order to destroy it. Steam under pressure, at a temperature of 115° C., killed it in half an hour; at 125° C. in five minutes. This extreme resisting power is exceptional, however, and many spores are destroyed in a few minutes by the boiling temperature of water.

In practice we assume that some of the more resistant spores, which are frequently present in the atmosphere, may have fallen into our culture material, and to insure its sterilization we subject it to a temperature which can be depended upon to destroy these; or we resort to the method of *discontinuous heating*. This method was first employed by Tyndall (1877), and is now in general use in the bacteriological laboratories of Germany, having been adopted by Koch and his pupils; while in France a single sterilization by means of steam under pressure, securing a higher temperature, is still the favorite method with many.

In the method by discontinuous heating we subject the culture material for a short time to the temperature of boiling water, thus destroying all bacteria in the vegetative stage. After an interval, usually of twenty-four hours, we repeat the operation for the purpose of destroying those which in the meantime have developed from spores which may have been present. Again the material is put aside, and after twenty-four hours it is again heated to the boiling point. This is usually repeated from three to five times. The object in view is to kill the growing bacteria which are developed from spores which were present; and, as a matter of experience, we find that this method of sterilization is more reliable than a single prolonged boiling, unless this be effected at a higher temperature than that of boiling water at the ordinary pressure of the atmosphere. Discontinuous heating is especially useful for the sterilization of liquids which would be injured by prolonged boiling—as is the case with solutions of gelatin—or which are coagulated by the boiling temperature. By means of a water bath, the temperature of which is regulated automatically, we may conduct the operation at any desired degree. Thus in sterilizing blood serum we use a temperature a little below that at which coagulation occurs (about 70° C.).

Test tubes, flasks, and apparatus of various kinds are commonly sterilized by dry heat in a *hot-air oven*. This is usually made of sheet iron, with double walls, and shelves for supporting the articles to be sterilized. The form shown in Fig. 23 is commonly used in bacteriological laboratories.

It must be remembered that a much higher temperature is required for the destruction of microörganisms when dry heat is employed than is the case with moist heat. The experiments of Koch and Wolffhügel (1881) show that a temperature of 120° to 128° C. (248° to 262° F.) is required to destroy the spores of mould fungi, and micrococci or bacilli in the absence of spores. For the spores of bacilli a temperature of 140° C. (284° F.), maintained for three hours, was required.

In practice we usually maintain a temperature of about 150° C. (302° F.) for an hour or more; and it is customary to sterilize all test tubes and flasks, which are to be used as receptacles for culture media, in the hot-air sterilizer. This procedure could no doubt, however, be dispensed with in many cases and reliance be placed upon the sterilization of the flask, together with its contents, in the steam sterilizer, especially with such culture media as are not injured by long exposure to a boiling temperature—*e.g.*, bouillon and agar-agar.

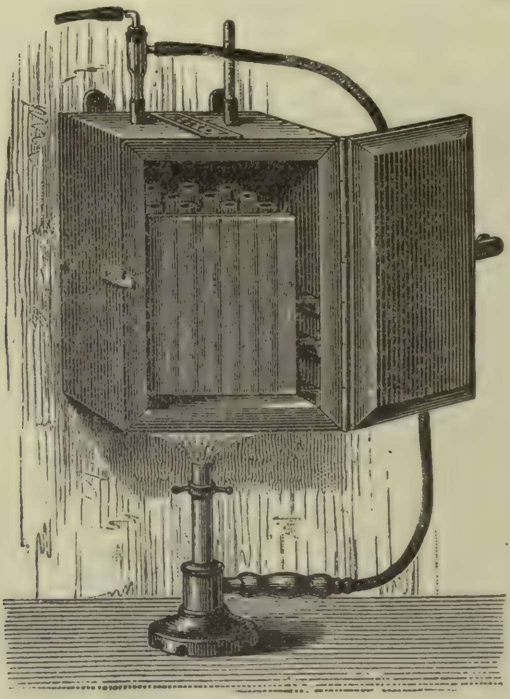

FIG. 23.

When we propose to cultivate aërobic bacteria, or such as require oxygen for their development, a cotton air filter is placed in the mouth of each test tube and flask before it is sterilized in the hot-air oven. This is a loose plug of cotton, pushed into the neck of the flask for an inch or more, and projecting from its mouth for a short distance. These cotton filters should fill the tube completely and uniformly, but should not be packed so closely that there is difficulty is removing them.

Steam Sterilizers.—Steam at the ordinary pressure of the atmosphere has the same temperature as boiling water, and in practice is preferable to a water bath for several reasons. The form of steam sterilizer adopted by Koch, after extensive experiments made in collaboration with Löffler and Gaffky, is now generally used in bacteriological laboratories. This is shown in Fig. 24. It consists of a cylindrical vessel of zinc which is covered with a jacket of felt. The cover, also covered with non-conducting material, has an aperture at the top for the escape of steam. A glass tube, which is in communication with the interior of the vessel, serves to show the height of the water when the apparatus is in use. The bottom of the cylindrical vessel should be of copper. A Bunsen burner having three jets will commonly be required to keep the water in ebullition and the upper part of the steam sterilizer filled with "live steam," which should escape freely from the aperture in the cover to insure a temperature of 100° C. in the steam chamber. A perforated zinc or copper shelf in the interior of the cylinder serves to support the flasks, etc., which are to be sterilized. Usually they are lowered into the cylinder in a light wire basket, or tin pail with perforated bottom, of proper diameter to slip easily into the sterilizer.

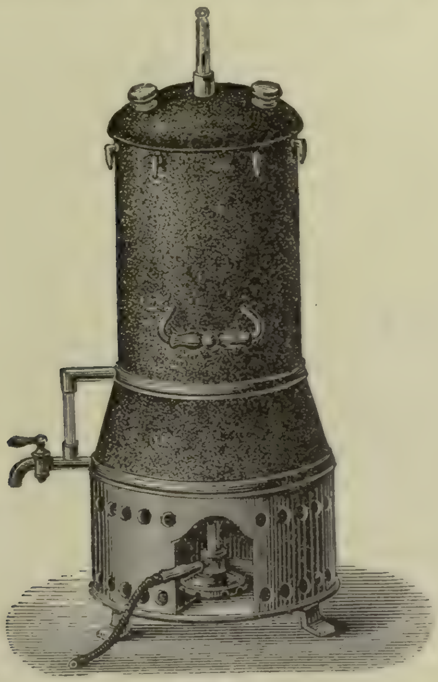

FIG. 24.

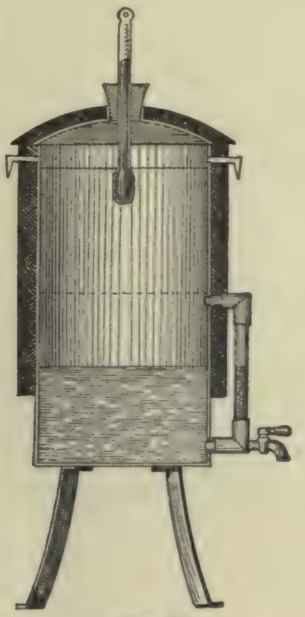

FIG. 25.

Fig. 25 is a sectional view of this sterilizer.

The steam sterilizer shown in Fig. 26 [1] is an American invention, which answers the purpose admirably, and which has the advantage of getting up steam very quickly and also of using comparatively little gas.

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[1] The Arnold steam sterilizer, manufactured at Rochester, N. Y.

The use of steam under pressure, by which higher temperatures are obtained, requires a more expensive apparatus, made on the principle of Papin's digester. The form manufactured by Müncke is one of the best. This is shown in Fig. 27. It is provided with a pressure gauge and a safety valve. A single sterilization in this apparatus, at a temperature of 115° C., for half an hour, will usually suffice, and for liquid culture media or for agar-agar this method is entirely satisfactory; but a gelatin medium which is exposed to this temperature loses its property of forming a jelly at 20° to 22° C., and consequently its value as a solid culture medium. In practice the simpler form of apparatus in which streaming steam is used will be found to answer every requirement. To insure sterilization with this it is customary to resort to discontinuous heating, as heretofore described. The standard flesh-peptone-gelatin medium should, as a rule, be subjected to a temperature of 100° C. for ten minutes, at intervals of twenty-four hours, four days in succession. Bouillon, flesh infusions, and agar-agar jelly may be steamed for an hour at a time two or three days in succession.

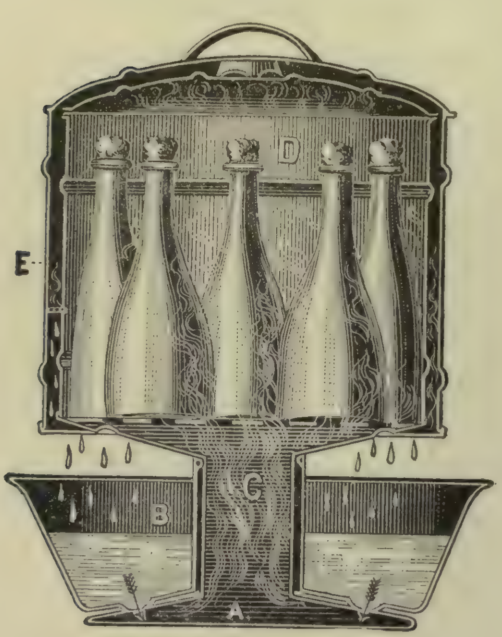

FIG. 26.

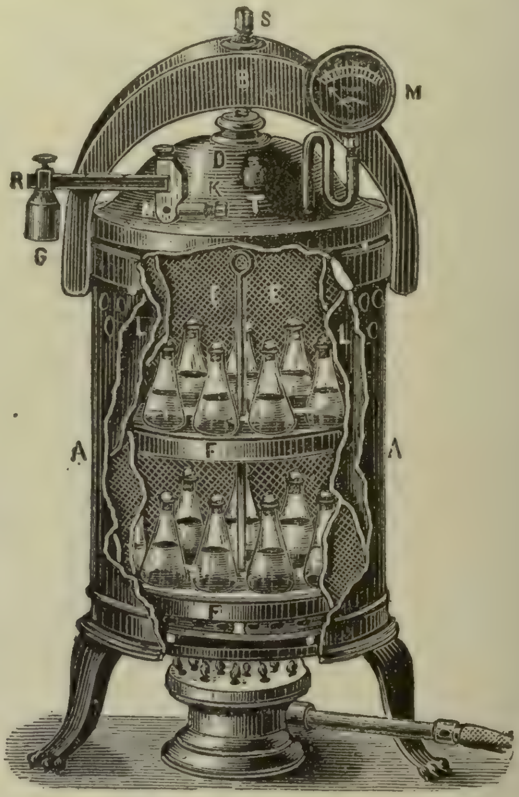

FIG. 27.

It is always advisable to test the sterilization of culture material before making use of it. This is done by placing it for a few days in an incubating oven at 30° to 35° C. If a considerable quantity of material in test tubes has been prepared at one time, it will be sufficient to put a few tubes in the incubating oven to test sterilization.

Failure to make this test often leads to serious complications in experimental investigations. A laboratory sometimes becomes infected with resistant spores, which are not all destroyed by the usual methods of sterilization, and these may not develop until some time has elapsed after the supposed sterilization.

Sterilization of Blood Serum.—Blood serum which has been collected in test tubes or small flasks, as heretofore directed, is sterilized in a water bath at 60° C. (140° F.) by the method of discontinuous heating. It is usually left in the hot-water bath for about an hour, and this is repeated, at intervals of twenty-four hours, for five to seven days. This rather tedious process may be avoided by collecting the serum in the first instance with proper precautions to prevent it from becoming contaminated with atmospheric organisms. A special apparatus was devised by Koch for sterilizing blood serum, but an improvised hot-water bath which is regulated to a temperature of 60° C. by an automatic thermo-regulator will answer the purpose. After being sterilized the serum is solidified by careful exposure to a temperature of about 68° C., which causes it to coagulate, forming a transparent, jelly-like mass. When coagulated at a higher temperature it becomes opaque. The time required for this operation varies from half an hour to an hour, and it is best to remove the tubes from the receptacle in which they are exposed to

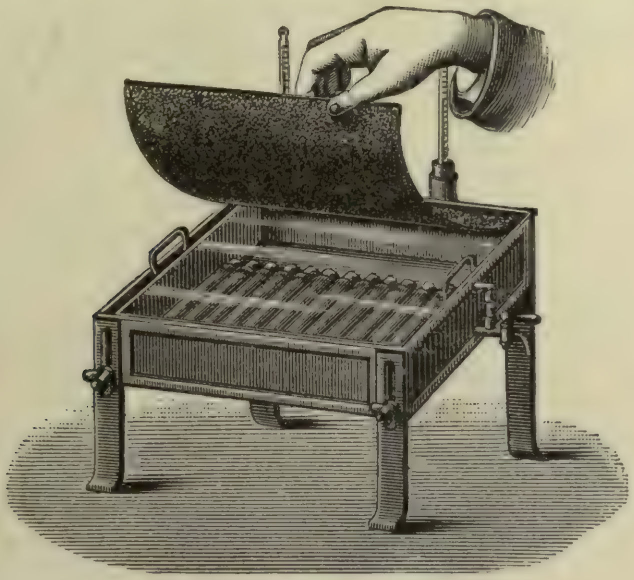

FIG. 28.

heat as soon as the serum is solidified. Koch's apparatus for coagulating blood serum is shown in Fig. 28. It is customary to place the test tubes in an oblique position, so that a large surface may be exposed upon which to cultivate the tubercle bacillus or whatever microörganism may be under investigation. A form of apparatus designed for both sterilizing and coagulating blood serum is shown in Fig. 29. It is manufactured by Müncke in accordance with the directions of Hueppe, and special precautions have been taken to secure a uniform temperature in all parts of the air chamber. We may remark that since it has been shown by Roux and Nocard that the tubercle bacillus grows very well in agar-agar jelly to which five per cent of glycerin has been added, blood serum is not so largely used as a culture medium in bacteriological laboratories.

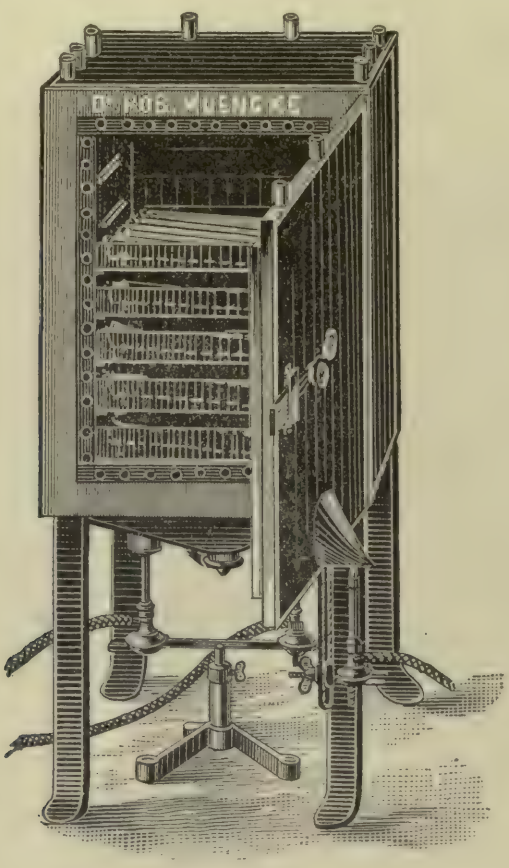

Fig. 29.

Sterilization by Filtration.—This method is especially useful for separating the soluble substances contained in a liquid culture of bacteria from the living cells. It has been demonstrated that several of the most important pathogenic bacteria produce toxic substances during their growth which may cause the death of susceptible animals independently of the living bacteria; and this demonstration has been made either by sterilizing a pure culture by means of heat, or by separating the bacteria from the culture-liquid by filtration. Some of these toxic products of bacterial growth are destroyed by a comparatively low temperature; the method of sterilization by filtration is therefore very important in researches relating to the composition and pathogenic power of these soluble products. Pasteur, in his earlier experiments, used plaster of Paris as a filter, and subsequently resorted to the use of unglazed porcelain, through which a liquid may be forced by pressure, but which does not permit of the passage of suspended particles, however small.

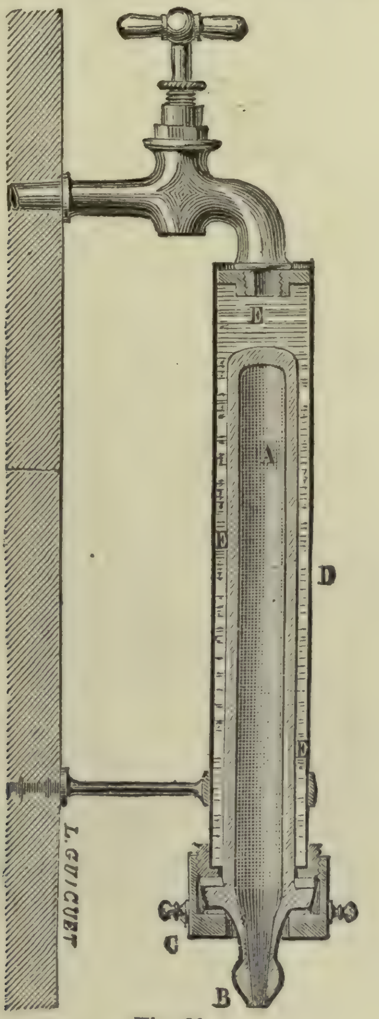

Fig. 30.

As the porcelain filter is the most reliable and convenient for accomplishing the object in view, we shall not describe other methods of filtration which have been proposed and successfully used. The porcelain used is a very fine paste, manufactured at Sèvres, which is moulded into cylinders (*bougies*) of the form proposed by Chamberland and baked at a high temperature.

In Fig. 30 the Pasteur-Chamberland filter is shown as arranged for the filtration of water. A is the hollow porcelain cylinder, which is enclosed in a metal case, D. The metal case is tightly clamped against a projecting shoulder at the lower part of the porcelain filter, a ring of rubber being interposed to secure a tight joint. When water under pressure is admitted to the space E, between the cylinder of porcelain and the metal case, it slowly filters through, and, running down the inner wall of the filter, escapes at B into a receptacle placed to receive it. If we fill the space E with a liquid culture of bacteria and apply sufficient pressure (one or two atmospheres), a clear filtrate is obtained which is entirely sterile if the porcelain filter is sound and made of proper material. After the filter has been in use for some time, however, it may permit the passage of bacteria, and it will be necessary to subject it to a high temperature for the purpose of destroying all organic matter contained in the porous porcelain.

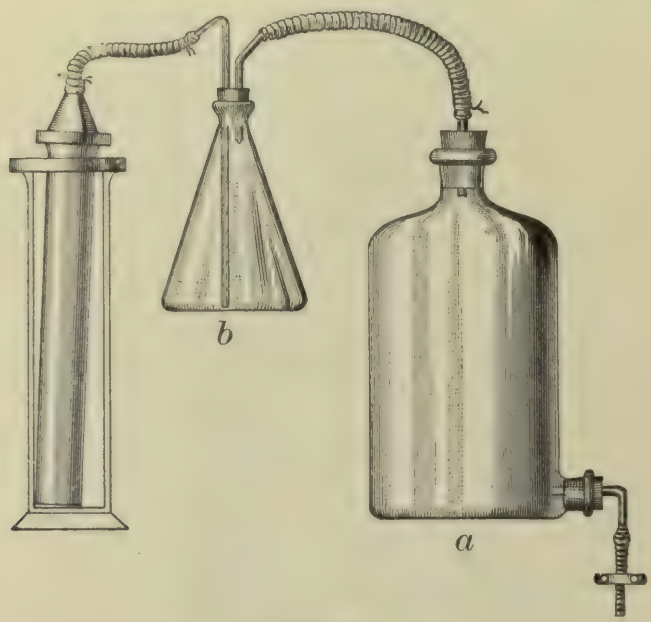

FIG. 31.

We may use the Chamberland filter without a metal case by immersing it in a cylindrical glass vessel containing the liquid to be filtered, as shown in Fig. 31. The porcelain cylinder is connected with an aspirator bottle, *a*, and a small Erlenmeyer flask, *b*, is interposed to catch the filtrate when it overflows from the interior of the filter. Of course all the necessary precautions must be taken with reference to the sterilization of the interior of the *bougie*, of the flask *b*, and of the rubber tube connecting the two.

Another arrangement of the Pasteur-Chamberland filter for laboratory purposes is shown in Fig. 32. In this form of apparatus a receptacle, R, is provided for the liquid to be filtered, and a pump for compressing air is attached to it by a rubber tube. Instead of this pump, water pressure may be used indirectly by attaching a strong bottle to the water supply and allowing it to fill slowly with water, and at the same time to force out the air through a tube connected with the filtering apparatus. For this purpose the bottle, having a capacity of a quart or more, should be provided with a rubber stopper through which two short tubes are passed. One of these is connected with the water supply and the other with the filter. Of course this is only practicable when a water supply with sufficient pressure is available.

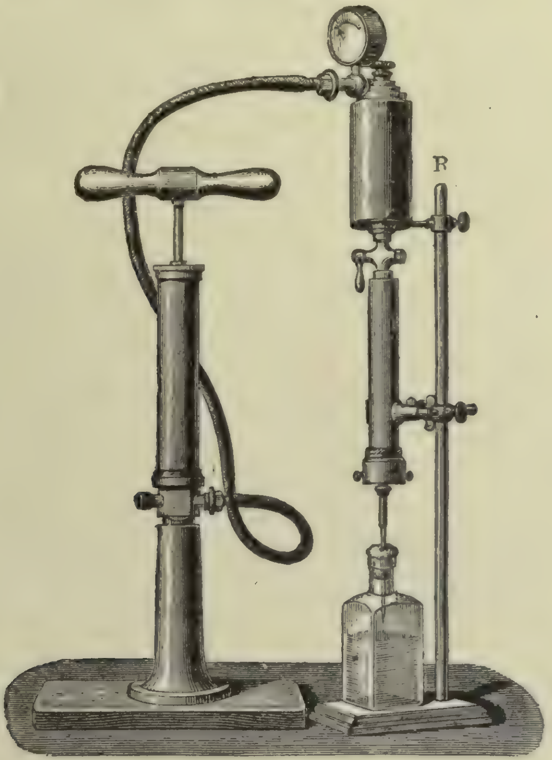

FIG. 32.

As a rule, filtration cannot be substituted with advantage for sterilization by heat in the preparation of culture media. Albuminous liquids pass through the filter with difficulty, and the process of sterilization by discontinued heating will usually prove more satisfactory than filtration, which requires extreme precautions to prevent accidental contamination of the filtered liquid. Moreover, the filter may change the composition of the medium passed through it by preventing the passage of colloid and albuminous material in solution. Thus, in an attempt to separate blood corpuscles from the serum by filtration through a Chamberland filter, the writer obtained a transparent liquid which did not coagulate by heat—*i.e.*, the albuminous constituents of the serum did not pass through the filter.

VII.

CULTURES IN LIQUID MEDIA.

PRIOR to the introduction of gelatinous media by Koch in 1881, cultures were made in various organic liquids, and these are still largely used, being for certain purposes preferable to solid media. The method of preparing and sterilizing the flesh infusions and other organic liquids commonly used has already been given. We are here concerned with the various modes of using these nutritive liquids in cultivating bacteria.

Flasks and tubes of various forms have been employed by different investigators, but the most useful receptacle for liquid as well as for solid culture media is *the ordinary test tube.* These are carefully cleaned, plugged with a cotton air filter, sterilized in the hot-air oven at 150° C., and are then ready to receive the filtered liquid. Usually the tube should not be filled to more than one-third to one-half of its capacity. Sterilization of the culture liquid is then effected by placing the tubes in the steam sterilizer for half an hour on three successive days. Before using, the tubes should be placed for a few days in an incubating oven at 30° to 35° C. to test the sterilization. This is especially important with liquid media, for if a single living spore is present it may give rise to an abundant progeny, which will be distributed through the liquid in association with the species which has been planted. In solid cultures, on the contrary, such a spore would give rise to a colony, which by its locality and characters of growth would probably be recognized as different from the species planted, and consequently accidental. This is the great danger in the use of liquid media; imperfect sterilization, or accidental contamination by atmospheric germs, may lead the inexperienced student into serious errors resulting from the assumption that the microörganisms present in his cultures are all derived from the seed he planted.

On the other hand, liquid media are more convenient than solid when it is the intention to isolate by filtration the soluble products of bacterial growth; for injection into animals to test pathogenic power; for experiments on the germicidal or antiseptic power of chemical agents, etc.

For larger quantities of liquid than can be held in an ordinary test tube the small flasks with a flat bottom, known as Erlenmeyer flasks, are very convenient (Fig. 33).

In his earlier researches Pasteur used flasks and tubes of various forms, which served a useful purpose, but have been displaced in his laboratory by the simpler form of apparatus shown in Fig. 34. This is a little flask having a cover which is ground to fit the neck. This cover is drawn out above into a narrow tube which admits oxygen to the flask through a cotton air filter. To obtain access to the interior of the flask for the purpose of introducing bacteria to start a culture, or to obtain material for microscopical examination, the cover is detached at the ground joint by a gentle twisting motion.

There is much less danger that a sterile culture liquid will become contaminated during the momentary removal of the cover from one of these little flasks, or of the cotton plug from a test tube, than is usually supposed. Abundant laboratory experience demonstrates that such contamination by bacteria floating in the atmosphere rarely occurs. The spores of mould fungi are commonly more abundant in the air, but even these do not very frequently fall into the culture liquid when the tube is opened to inoculate it with the bacteria it is proposed to cultivate. This inoculation is best made with a platinum wire, bent into a loop at the free extremity, and sealed fast into the end of a glass rod (Fig. 35). This is sterilized in the flame of a Bunsen burner or alcohol lamp by bringing the platinum wire to a red heat and passing the end of the glass rod which carries it through the flame several times. With this instrument we may transfer a little drop from a culture to the sterile fluid in another

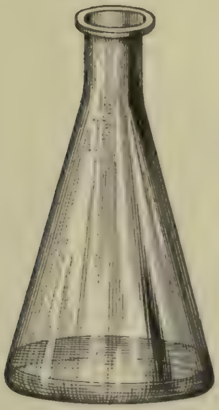

FIG. 33.

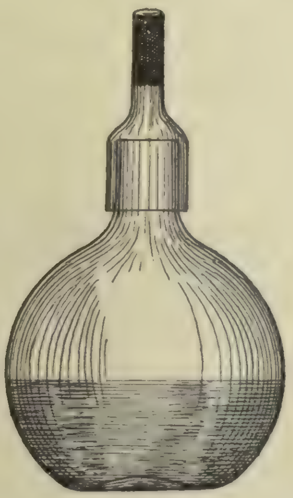

FIG. 34.

tube for the purpose of starting a new culture. Or we may start a pure culture from a drop of blood taken from the veins of an animal which has been inoculated with anthrax, or any similar infectious disease in which the blood is invaded by a bacterial parasite.

But if we have not a pure culture to start with our liquid media do not afford us the means of obtaining one; and if two or more bacteria which resemble each other in their morphology are associated in such a culture we cannot differentiate them, and are likely to infer that we have a pure culture of a single microörganism when this is not really the case.

But if we have pure stock to start with we may maintain pure cultures in liquid media without any special difficulty.

Various *characters of growth*, etc., are to be observed in cultivating different microörganisms in liquid media. Thus some grow at the surface in the form of a thin film or membranous layer—"mycoderma"—while others are distributed uniformly through the liquid, rendering it opalescent or more or less milky and opaque; others, again, form little flocculi which are suspended in the transparent fluid. Usually, when active growth has ceased, the bacteria fall to the bottom of the tube as a more or less abundant, white or colored, pulverulent or glutinous deposit. In some cases the liquid is colored with a soluble pigment formed during the growth of the bacteria, and usually this is formed most abundantly at the surface, where there is free access of oxygen. The reaction of the medium is often changed as a result of the growth of bacteria in it. From being neutral it may become decidedly alkaline or acid in its reaction. These changes may be observed by adding a litmus solution before sterilization of the culture medium, and observing the change of color when an acid-producing bacterium is under cultivation. The reducing power of bacteria upon various aniline colors may also be studied; also their power to break up various organic substances, as shown by the evolution of gas or other volatile products which may be collected, or by substances which remain in solution and can be studied by ordinary chemical methods.

FIG. 35.

Drop Cultures.—When we desire to study the life history of a microörganism and to witness its development from spores, for example, its motions, etc., the method of cultivation in a hanging drop of culture fluid, attached to a thin glass cover and suspended over a circular excavation ground out of a glass slide, is very useful. Such a drop culture may be left under the microscope and kept under observation for hours or days.

In making these drop cultures it is necessary to sterilize the glass slides and thin glass covers by heat, and to take every precaution to prevent the inoculation of the drop of culture liquid with any other bacteria than those which are to be studied.

The simplest form of moist chamber for drop cultures consists of an ordinary glass slide having a concave depression, about fifteen millimetres in diameter, ground out in its centre. This and the thin glass cover, having been sterilized by exposure in the hot-air oven at 150° C. for an hour or more, or by passing them through the flame of an alcohol lamp, are ready for use. The cover glass is held in sterile forceps, and a little drop of the culture fluid containing the bacterium to be studied is transferred to its centre by means of the platinum loop heretofore described. It is best to spread the drop out as thin as possible, and it may be inoculated, from a pure culture, with a platinum needle (Fig. 36) after it has been placed upon the cover. This is then inverted over the hollow place in the glass slide, and it is customary to prevent the entrance of air and attach the cover by spreading a little vaseline around the margin of the excavation.

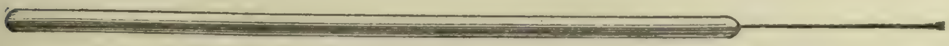

Fig. 36.

Another form of moist chamber is made by attaching a glass ring, having parallel, ground surfaces, to the centre of a glass slide by a suitable cement.

In Ranvier's moist chamber there is a central eminence surrounded by a groove ground into the glass slide, and the drop of culture fluid is in contact with a polished glass surface below as well as above. This affords a more satisfactory view under the microscope.

The Author's Culture Method.—In a paper read at the meeting of the American Association for the Advancement of Science, in August, 1881, the writer described a method of conducting culture experiments which he has since used extensively and with very satisfactory results. The liquid culture medium is preserved in little flasks having a long neck which is hermetically sealed. The principal advantages connected with the use of these little flasks, or "Sternberg's bulbs," as they are sometimes called, are that a culture medium may be preserved in them indefinitely and that they are easily transported from place to place; whereas test tubes, Pasteur's flasks, and similar receptacles must be kept upright, and after a time the culture liquid in them is changed in its composition by evaporation. They are also liable to be contaminated by the entrance of mould fungi when kept in a damp place. The spores of these fungi, falling upon the surface of the cotton air filter, germinate, and the mycelium grows down through the cotton into the interior of the tube, where a new crop of spores is quickly formed. It is, therefore, a convenience to have sterile culture liquids always ready for use in a receptacle which can be packed in a box and transported from place to place; but for every-day use in the laboratory the ordinary test tube, with its cotton air filter, is the most economical and convenient receptacle for culture liquids as well as for solid media. With reference to the method of making and using these little flasks, I quote from a paper published in the *American Journal of the Medical Sciences* in 1883 :[1]

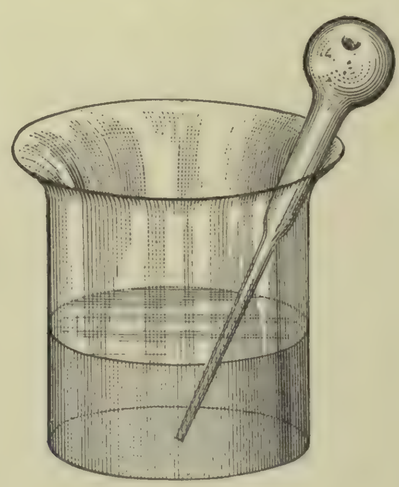

FIG. 37.

> The culture flasks employed contain from one to four fluidrachms. They are made from glass tubing of three- or four tenths inch diameter, and those which the writer has used in his numerous experiments have all been "home-made." It is easier to make new flasks than to clean old ones, and they are thrown away after being once used. Bellows operated by foot, and a flame of considerable size—gas is preferable—will be required by one who proposes to construct these little flasks for himself.[2] After a little practice they are made rapidly; but as a large number are required, the time and labor expended in their preparation are no slight matter. After blowing a bulb at the extremity of a long glass tube, of the diameter mentioned, this is provided with a slender neck, drawn out in the flame, and the end of this

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[1] "The Germicide Value of Certain Therapeutic Agents," op. cit., vol. clxx.

[2] A glass-blower ought to make them for two or three dollars per hundred.

is hermetically sealed. Thus one little flask after another is made from the same piece of tubing until this becomes too short for further use. To introduce a culture liquid into one of these little flasks, heat the bulb slightly, break off the sealed extremity of the tube and plunge it beneath the surface of the liquid (Fig. 37). The quantity which enters will of course depend upon the heat employed and the consequent rarefaction of the enclosed air. Ordinarily the bulb is filled to about one-third of its capacity with the culture liquid, leaving it two-thirds full of air for the use of the microscopic plants which are to be cultivated in it. . . . Sterilization is effected by heat after the liquid has been introduced and the neck of the flask hermetically sealed in the flame of an alcohol lamp.

Sterilization may be effected by boiling for an hour in a bath of paraffin or of concentrated salt solution, by which a temperature considerably above that of boiling water is secured. The writer is in the habit of preparing a considerable number of these flasks at one time, and leaving them, in a suitable vessel filled with water, for twenty-four hours or longer on the kitchen stove.[1]

To inoculate the liquid contained in one of these little flasks with microörganisms from any source, the end of the tube is first heated to destroy germs attached to the exterior; the extremity is then broken off with sterilized (by heat) forceps; the bulb is very gently heated, so as to force out a little air, and the open end is plunged into the liquid containing the organism to be cultivated (or into a vein, or one of the solid viscera of an animal dead from an infectious germ disease, such as anthrax).

Inoculation from one tube to another may also be effected by means of the ordinary platinum wire needle.

Before the introduction of Koch's plate method for isolating bacteria in pure cultures, certain methods had been proposed, and employed to some extent, which at present have a historical value only.

Thus Klebs (1873) proposed to take from a first culture in which two or more species were associated a minute quantity, by means of a capillary tube, and with this to inoculate a second culture. By repeating this procedure several times he expected to exclude all except the species which was present in the greatest abundance and which multiplied most rapidly in the medium employed.

The *method by dilution*, first employed with precision by Brefeld (1872) in obtaining pure cultures of mould fungi, and subsequently by Lister for the isolation of bacteria, consists in so diluting a minute quantity of the mixed culture that the number of bacteria in the dilution may be less than one for each drop of the liquid. If now a single drop be added to each of a series of tubes containing a small quantity of sterile bouillon, some of the inoculations made may give a pure culture, as the drop may have contained but a single vegetative cell.

Another method of obtaining a pure culture in liquid media, when several microörganisms are associated which have a different thermal death-point, consists in the application of heat and thus destroying all except the most resistant species. This method is especially applicable when one of the species, only, forms spores. By subjecting the mixed culture to a temperature which is sufficient to destroy all the vegetative cells in it, the more resistant spores are left and, under favorable conditions, may subsequently vegetate and give us a pure culture of the species to which they belong.

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[1] Where a steam sterilizer is at hand they will be most conveniently sterilized in the usual way, by subjecting them to the boiling temperature for an hour at a time on three successive days.

Fermentation.—The development of certain bacteria is attended with an evolution of gas, especially in media containing grape sugar or glycerin. For the determination of the quantity and kind of gas produced by a given micro-organism the fermentation tube recommended by Theobald Smith has special advantages. This is a bent tube (Eihorn's) supported upon a glass base as shown in the accompanying figure taken from the catalogue of Eimer & Amend. The graduation shown upon the upright arm is not essential for ordinary laboratory work. A liquid culture medium containing one to two per cent of grape sugar is usually used. This is introduced into the upright arm of the fermentation tube, where it is held by atmospheric pressure. A cotton plug is placed in the opening of the short and bulbous arm of the tube, which is intended as a receptacle for the culture liquid when it is forced out of the closed arm by the accumulation of gas at its upper extremity.

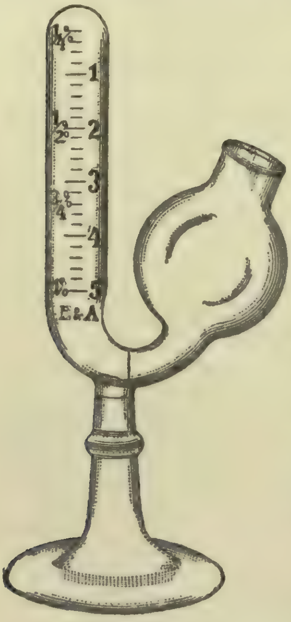

FIG. 38.

VIII.

CULTURES IN SOLID MEDIA.

THE introduction of solid culture media in 1881 by the famous German bacteriologist, Robert Koch, inaugurated a new era in the progress of our knowledge relating to the bacteria. His methods enable us to obtain pure cultures with ease and certainty, and to study the morphological and biological characters of each species free from the complications which led to so much error and confusion before these methods were introduced. We have already given an account of the method of preparing and sterilizing the various solid culture media, and are here concerned with the manner in which they are used and the special advantages which they afford.

Koch's flesh-peptone-gelatin, which contains ten per cent of gelatin, is a transparent jelly which liquefies at from 22° to 24° C. It is a favorable culture medium for a great number of bacteria, and many species show definite characters of growth in this medium which serve to differentiate them. One of the most prominent of these characters depends upon the fact that some bacteria liquefy gelatin and others do not. This is made apparent when we make "stab cultures." This is the usual manner of inoculating a solid culture medium, and is illustrated in Fig. 39. A platinum needle, consisting of a piece of platinum wire inserted into a glass rod which serves as a handle, is passed through the flame of an alcohol lamp to sterilize it. When cooled, which occurs very quickly, the point is introduced into the material containing the bacteria to be planted in the gelatin medium. We may obtain our seed for a pure culture from a single colony, from another stab culture, from the blood of an infected animal, etc. The point of the needle is then carried into the sterilized jelly, as shown in the figure, care being taken to introduce it in the central line and in a direction parallel with the sides of the tube. It is best

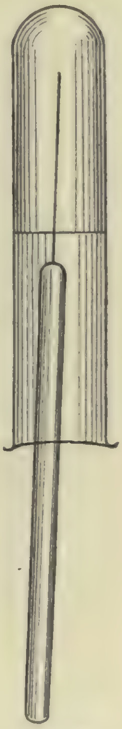

FIG. 39.

always to hold the tube inverted during the inoculation, and not to remove the cotton air filter until we are ready to make it. The cotton plug is then returned to its place and the platinum needle again brought to a red heat to destroy any bacteria which remain attached to it.

Sometimes it is an advantage to have the culture medium with a sloping surface, as shown in Fig. 40. We may then draw the needle over the surface in a longitudinal direction, and by this means distribute the seed in a line along which development will take place.

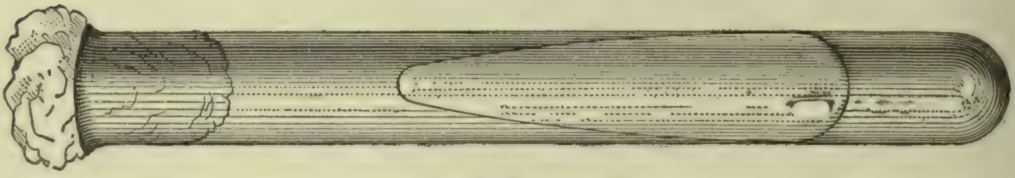

FIG. 40.

The characters of growth in these stab cultures in gelatin are very various. Non-liquefying bacteria may grow only on the surface, as at *a*, Fig. 40A; or both on the surface and along the line of puncture, as at *b;* or only at the bottom, as at *c*. In the first case the microörganism is *aërobic*—that is, it requires oxygen, and grows only in the presence of this gas. In the second case it is not strictly aërobic, but may grow either in the presence of oxygen

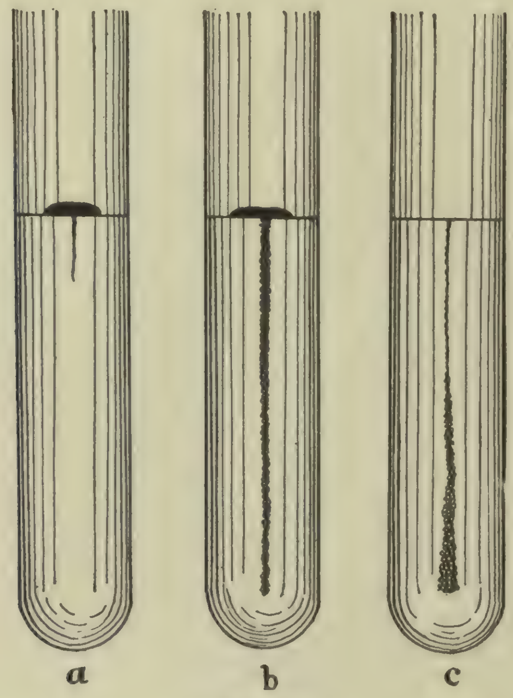

FIG. 40A.

or in its absence—a *facultative anaërobic.* In the third case the microörganism is an *anaërobic,* which cannot grow in the presence of oxygen, and consequently does not grow upon the surface of the culture medium or along the upper portion of the line of puncture.

Again, we have differences as to the character of growth upon the surface or along the line of puncture. The surface growth may be a little mass piled up at the point where the needle entered the gelatin; or it may form a layer over the entire surface, and this may be thin or thick, dry or moist, viscid or cream-like, and of various colors—green, blue, red, or yellow, of different shades—or more frequently of a milk-white color.

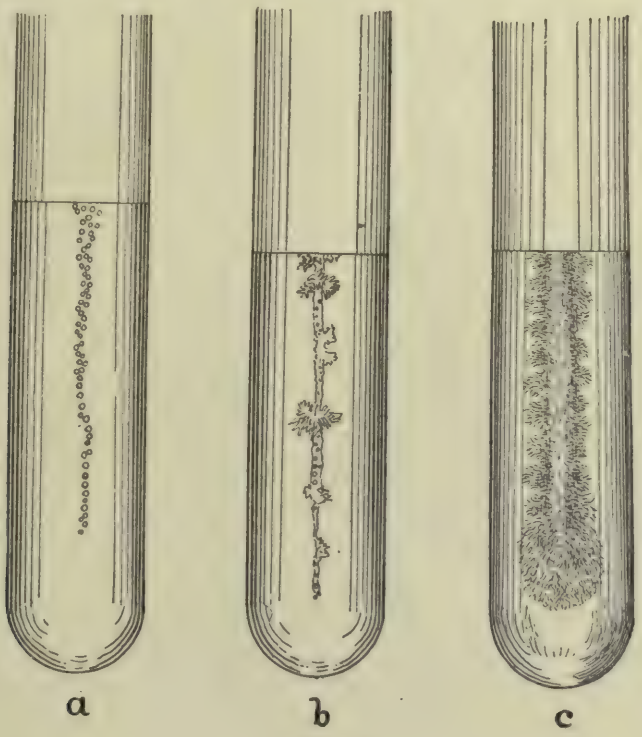

FIG. 41.

The growth along the line of puncture also differs greatly with different species. We may have a number of scattered spherical colonies (*a*, Fig. 41), and these may be translucent or opaque; or we may have little tufts, like moss, projecting from the line of puncture (*b*, Fig. 41); or slender, filamentous branches may grow out into the gelatin (*c*, Fig. 41).

The liquefying bacilli also present different characters of growth. Thus liquefaction may take place all along the line of puncture, forming a long and narrow funnel of liquefied gelatin (*a*, Fig. 42); or we may have a broad funnel, as at *b*; or a cup-shaped cavity, as at *c*; or the upper liquefied portion may be separated from that which is not liquefied by a horizontal plane surface, as at *d*.

The characters of growth in agar-agar jelly are not so varied, but this medium possesses the advantage of not liquefying at a temperature of 35° to 38° C., which is required for the development of certain pathogenic bacteria. Variations in mode of growth are also manifested in nutrient agar similar to those referred to as produced by non-liquefying bacteria in flesh-peptone-gelatin. These relate to the surface growth and to growth along the line of puncture. One character not heretofore mentioned consists in the formation of gas bubbles in stab cultures either in gelatin or agar.

Colonies.—If we melt the gelatin or agar in a test tube, pour the liquid medium into a shallow glass dish previously sterilized, and allow it to cool while properly protected by a glass cover, we will have a broad surface of sterile nutrient material. If now we expose it to the air for ten or fifteen minutes, and again cover it and put it aside for two or three days at a favorable temperature, we can scarcely fail to have a number of colonies upon the surface of the culture medium, which have been developed from atmospheric germs which were deposited upon it during the exposure. Each of these colonies, as a rule, is developed from a single bacterium or spore, and consequently the little mass, visible to the naked eye, which we call a colony, is a pure culture of a particular species. In this experiment we are more apt to have colonies of mould fungi than of bacteria, but the principle is the same, viz., that a colony developed from a single germ is a pure culture. By touching our platinum

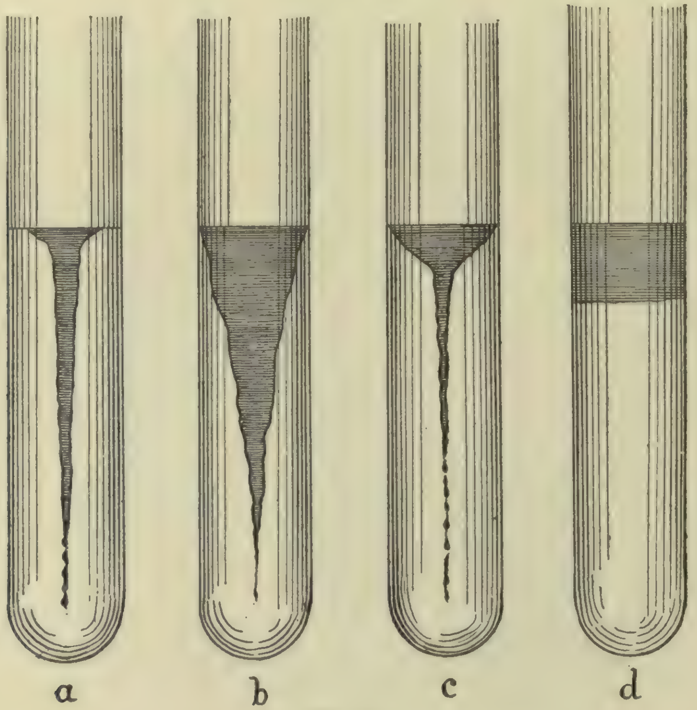

FIG. 42.

needle, then, to such a colony, which is quite independent of, and well separated from, all others, we may make a stab culture in gelatin or agar, and preserve the pure culture for further study. This is a most important advantage which pertains to the use of solid culture media. It is a singular fact that, as a rule, colonies of bacteria which lie near each other do not grow together, but each remains distinct. If there are but few colonies, each one, having plenty of room, may grow to considerable size ; if there are many and they are crowded, they remain small, but are still independent colonies.

Now, these colonies differ greatly in their appearance and characters of growth, according to the species (Fig. 43). Some are spherical, and these may be translucent or opaque, or they may have an opaque nucleus surrounded by a transparent zone. Again, the outlines may be irregular, giving rise to amœba-like forms, or to a fringed or plaited margin, or the form may be that of a rosette, etc. ; or the colony may appear to be made up of overlapping scales or masses, or of tangled filaments ; or it may present a branching growth. In the case of liquefying bacteria, when the colonies have developed in a gelatin medium they commonly do not at once cause liquefaction of the gelatin, but at the end of twenty-four hours or more the gelatin about them commences to liquefy and they are seen in a little funnel of transparent liquefied gelatin ; or in other cases little opaque drops of liquefied gelatin are seen, which, as the liquefaction extends, run together. All of these characters are best studied under a low-power lens, with an amplification of five to twenty diameters ; and by a careful observation of the differences in the form and development of colonies we are greatly assisted in the differentiation of species.

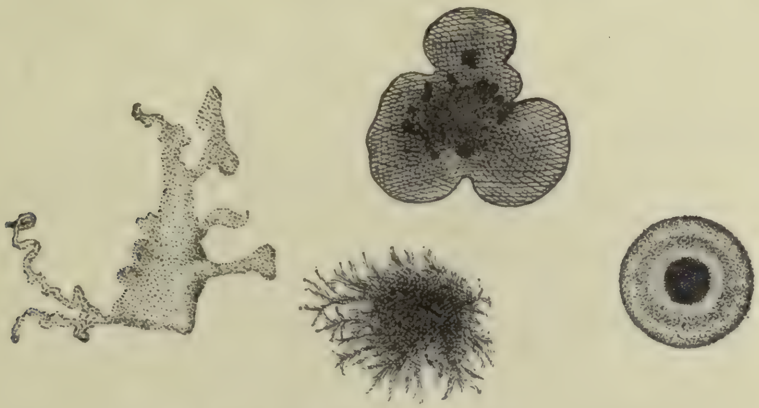

FIG. 43.—Colonies of Bacteria.

Single, *isolated colonies do not always contain a single species,* for they are not always developed from a single cell. We may have deposited upon our plate, exposed as above described, a little mass of organic material containing two or more different bacteria, and this would serve as the nucleus of a colony from which we could not obtain a pure culture.

Koch's Plate Method.—In the experiment above described, colonies were obtained from air-borne germs which were deposited upon the surface of our gelatin medium. By Koch's famous "plate method" we obtain colonies of any particular microörganism which we desire to study, or of two or more associated bacteria which we desire to study separately in pure cultures. Evidently, when we have obtained separate colonies of different bacteria upon the surface of a solid culture medium, we can easily obtain a pure culture of each by inoculating stab cultures from single colonies.

To obtain separate colonies we resort to the ingenious method of Koch. Three test tubes containing a small quantity of nutrient gelatin (or of agar) are commonly employed. The tubes are numbered 1, 2, and 3. The first step consists in liquefying the nutrient jelly by heat, and it will be well for beginners to place the tubes in a water bath having a temperature of about 40° C. (104° F.) for the purpose of keeping the culture material liquid, and at the same time at a temperature which is not high enough to destroy the vitality of the bacteria which are to be planted. We next, by means of a platinum-wire loop or the platinum needle used for stab cultures, introduce into tube No. 1 a small amount of the culture, or material from any source, containing the bacteria under investigation. Care must be taken not to introduce too much of this material, and it must be remembered that the smallest visible amount may contain many millions of bacteria. The reason for using three tubes will now be apparent. It is usually impossible to introduce a few bacteria into tube No. 1, but we effect our object by dilution, as follows: With the platinum-wire loop we take up a minute drop of the fluid in tube No. 1, through which the bacteria have been distributed by stirring, and carry it over to tube No. 2. Washing off the drop by stirring, we may repeat this a second or third time—this is a matter of judgment and experience; often it will suffice to carry over a single *öse* (the German name for the platinum-wire loop). Next we carry over one, or two, or three öse from tube No. 2 to tube No. 3. By this procedure we commonly succeed in so reducing the number of bacteria in tube No. 3 that only a few colonies will develop upon the plate which we subsequently make from it; or it may happen that the dilution has been carried too far and that no colonies develop upon the plate made from this tube, in which case we are likely to get what we want from tube No. 2. The next step is to pour the liquid gelatin upon sterilized glass plates, which are numbered to correspond with the tubes. The plates used by Koch are from eight to ten centimetres wide and ten to twelve centimetres long. They must be carefully cleaned and sterilized in the hot-air oven, at 150° C., for two hours. They may be wrapped in paper before sterilization, or placed in a metal box especially made for the purpose. In order that the liquid gelatin may be evenly distributed upon the plate the apparatus shown in Fig. 44 is used. This consists of a glass plate, *g*, supported by a tripod having adjustable feet. By means of the spirit level *l* the glass plate is adjusted to a horizontal position. A sterilized glass plate is placed in the glass tray, shown in the figure, and the gelatin from one of the tubes is carefully poured upon it and distributed upon its surface with a sterilized glass rod, care being taken not to bring it too near the edge of the plate. The glass tray in then covered until the gelatin has cooled sufficiently to become solid, after which plate No. 1 is removed and plates Nos. 2 and 3 are made in the same way. In order to save time it is customary to fill the glass tray shown in the figure with ice water, to place a second glass support upon it, and upon this the sterilized glass plate upon which the liquid gelatin is poured. This is protected by a glass cover, as before, until the gelatin becomes solid.

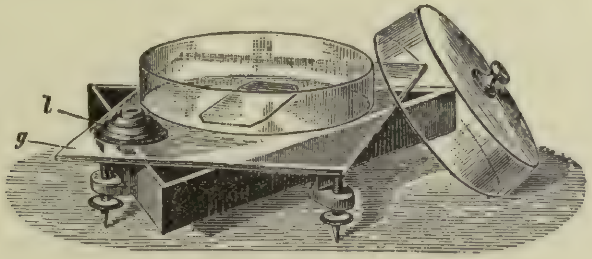

FIG. 44.

The three plates, prepared as directed, are put aside in a glass jar of the form shown in Fig. 44, one being supported above the other by a bench of sheet zinc or glass.

Petri's Dishes.—A modification of the plate method of Koch, which has some advantages, consists in the use of three small glass dishes of the same form as the larger one used by Koch to contain the plates. These dishes of Petri are about ten to twelve centimetres in diameter and one to 1.5 centimetres high, the cover being of the same form as the dish into which the gelatin is poured. These dishes take less room in the incubating oven than the larger glass jar used in the plate method, and they do not require the use of a levelling apparatus. The colonies also may be examined and counted, if desired, without removing the cover, and consequently without the exposure which occurs when a plate prepared by Koch's method is under examination.

In agar-agar cultures or in gelatin cultures of non-liquefying bacteria made in Petri's dishes, we may examine and count colonies, without removing the cover, by inverting the dish.

In pouring the liquefied gelatin from the test tubes in which the dilution has been made into sterilized Petri's dishes, care must be taken to first sterilize the lip of the test tube by passing it through the flame of a lamp. We may at the same time burn off the top of the cotton plug, then remove the remaining portion with forceps, when the lip has cooled, for the purpose of pouring the liquid into the shallow dish.

Von Esmarch's Roll Tubes.—Another very useful modification of Koch's plate method is that of von Esmarch. Instead of pouring the liquefied gelatin or agar medium upon plates or in shallow dishes, it is distributed in a thin layer upon the walls of the test tube containing it. This is done by rotating the tube upon a block of ice or in iced water. Esmarch first used a tray containing iced water, and to prevent the wetting of the cotton filter a cap of thin rubber was placed over the end of the tube. It is more convenient to turn the tubes upon a block of ice having a horizontal flat surface, in which a shallow groove is first made by means of a test tube containing hot water (Fig. 45). Or, in the winter, we may turn the tube under a stream of cold water from the city supply—*i.e.*, from a faucet in the laboratory. A little practice will enable the student to distribute the culture medium in a uniform layer on the walls of the test tube, and as soon as it is quite solidified these may be placed aside for the development of colonies from the bacteria which had been introduced. When roll tubes are made from the agar jelly it is best to place the tubes in a nearly horizontal position, for if placed upright at once the film of jelly is likely to slip from the walls of the

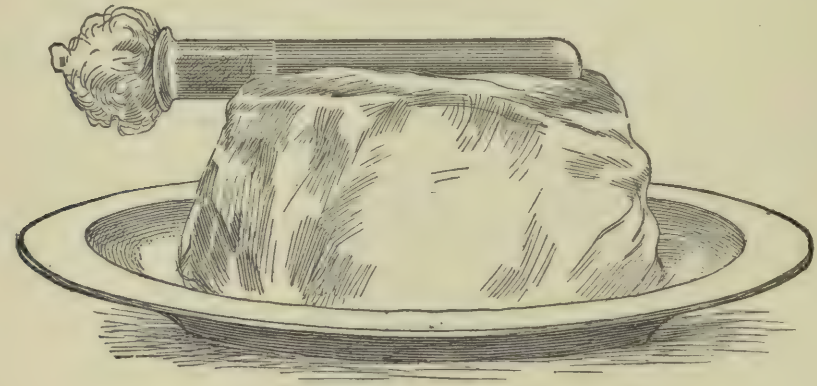

FIG. 45.

tube. This is due to the fact that a little fluid is pressed out of the jelly, probably by a slight contraction while cooling. If the tubes are slightly inclined from the horizontal the film does not slip and the fluid accumulates at the bottom. After a day or two they may be placed in an upright position.

These roll tubes possess several advantages. They are quickly made and take but little space in the incubating oven, and the film of jelly is protected from contamination by atmospheric germs. When colonies have formed we may examine them through the thin walls of the tube, either with a pocket lens or a low-power objective. In making a stab culture from a single colony in one of these roll tubes, we invert the tube, remove the cotton air filter, and pass the point of a sterilized platinum needle up to the selected colony. In the same way we obtain material for microscopical examination.

Streak Cultures.—In his earlier experiments with solid culture media Koch made "streak cultures" by drawing the point of a platinum needle, charged with bacteria, over the surface of a gelatin or agar plate; and this method is still useful in certain cases. If we draw the needle over the moist surface several times in succession the greater number of bacteria will be deposited in the first streak, and in the second or third single cells are likely to be left at such intervals from each other that each will develop an independent colony. If the streaks were made with impure stock we may thus succeed in getting separate colonies of the several bacteria contained in it, so that this method may be employed for obtaining pure cultures. But for this purpose it is much inferior to the plate method, and it is chiefly used for observing the growth of bacteria on the surface of solid culture media. Thus we commonly make a streak upon the surface of cooked potato or solidified blood serum in studying the development of various bacteria on these culture media.

Cultures upon Blood Serum.—The use of blood serum as a solid medium is practically restricted to stab cultures and streak cultures, for we cannot substitute it for the gelatin and agar media in making plates and roll tubes. This is because it only becomes solid at a temperature which would be fatal to most bacteria (70° C.), and when once made solid by heat cannot again be liquefied. Its use is, therefore, restricted mainly to the cultivation of bacteria for which it is an especially favorable medium. It may be used, however, in combination with a gelatin or agar medium. For this purpose it is most conveniently kept in a fluid condition in the little flasks heretofore described ("Sternberg's bulbs").

The gelatin or agar jelly in test tubes is liquefied by heat and cooled in a water bath to about 40° C. The desired amount of sterile blood serum is then forced into each tube by passing the slender neck of the little flask along the side of the cotton filter (see Fig. 46) and applying gentle heat to the bulb. The slender neck is first sterilized by passing it through a flame, and the point is broken off with sterile forceps. After inoculating the liquefied medium in the test tubes in the usual manner we may make plates or roll tubes.

Cultures on Cooked Potato.—The method of preparing potatoes for surface cultures has already been given (page 48). It was in using them that Koch first got his idea of the importance of solid media, which led to his introduction of the use of gelatin and agar-agar and the invention of the plate method. By means of streak cultures upon potato he had succeeded in obtaining isolated colonies and pure cultures. We now use the potato chiefly for the purpose of differentiating species. Some bacteria grow on the surface of cooked potato and some do not. Those which do present various characters of growth. Thus we have differences as to color, as to rapidity of growth, as to the character of the mass formed—thick or thin, viscid, moist or dry, restricted to line of inoculation or extending over the entire surface, etc.

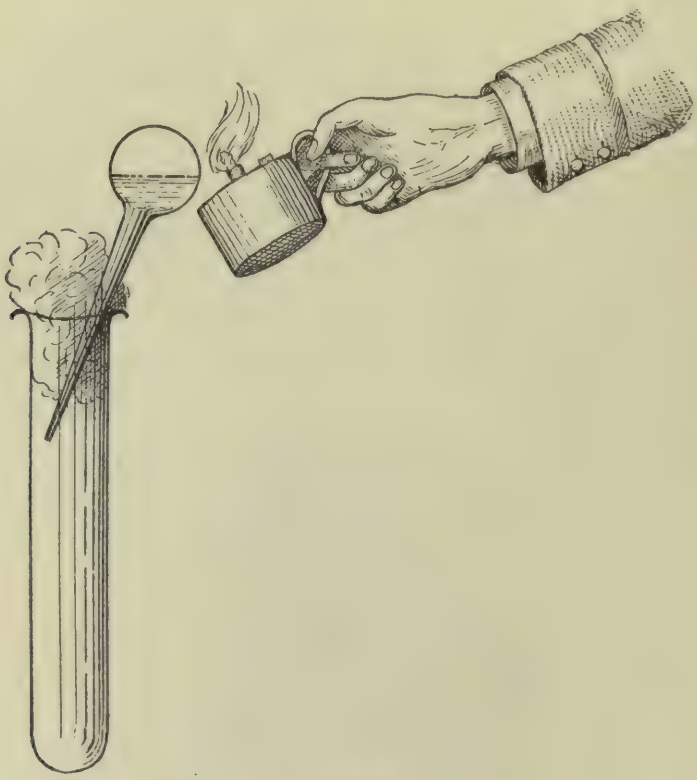

FIG. 46.

Instead of using a cut section of the potato in the manner heretofore described, we may make a *purée* by mashing the peeled and cooked tubers and distributing the mass in Erlenmeyer flasks. After thorough sterilization by steam the culture medium is ready for use. In the same way other vegetables, or bread, etc., may be used for special purposes, and especially for cultures of the mould fungi.

Potatoes usually have a slightly acid reaction, and on this account certain bacteria will not grow upon them. This acid reaction is not constant and differs in degree, and as a result we may have decided differences in the growth of the same species upon different potatoes. To overcome this objection the writer has sometimes neutralized the cones of potato in test tubes (see Fig. 21, page 49) by first boiling them in water containing a little carbonate of soda. The liquid is poured off after they have been in the steam sterilizer for half an hour, and they are returned for sterilization.

Salomonson's Method of cultivation in capillary tubes has a historical value only since the introduction of Koch's plate method.

The following modifications of Koch's plate cultures have recently been introduced:

Kruse (1894) pours the liquefied gelatin or agar into Petri dishes, and after it is solidified brushes the surface with a sterilized camel's-hair brush which has been dipped into water containing in suspension—properly diluted—the bacteria to be studied. By this procedure surface colonies only are obtained. Von Freudenreich (1894) prefers to pour the contents of the test tube upon the surface of the sterile medium, in Petri dishes. The fluid is allowed to run off by placing the Petri dish in a vertical position, and this is subsequently placed in the incubating oven in an inverted position—*i.e.*, with cover below. To obtain satisfactory plates with well-separated, superficial colonies it may be necessary to use two or three dilutions, made in sterilized water in the usual way—*i.e.*, from one tube to another, by means of the platinum wire having a loop at its extremity.

IX.

CULTIVATION OF ANAËROBIC BACTERIA.

PASTEUR (1861) first pointed out the fact that certain species of bacteria not only grow in the entire absence of oxygen, but that for some no growth can occur in the presence of this gas. Such bacteria are found in the soil, and in the intestines of man and the lower animals. The cultivation of "strict anaërobics" calls for methods by which oxygen is excluded. The "facultative anaërobics" grow either in the presence or absence of oxygen. There are various gradations in this regard, from the strictly aërobic species which require an abundance of oxygen and will not grow in its absence, to the strictly anaërobic species which will not grow if there is a trace of oxygen in the medium in which we propose to cultivate them. Among the most interesting pathogenic bacteria which are strictly anaërobic are the bacillus of tetanus, the bacillus of malignant œdema, and the bacillus of symptomatic anthrax.

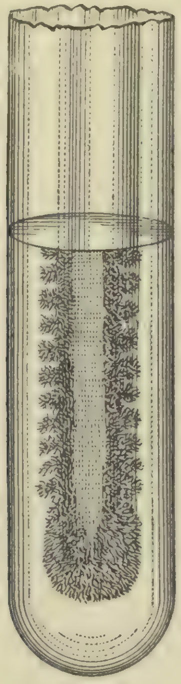

FIG. 47.

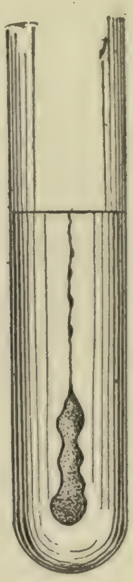

FIG. 48.

If we make an inoculation of one of the species which is not strictly anaërobic into a test tube containing nutrient gelatin or agar-agar, we may have a development all along the line of puncture, and this may be more abundant below, as in Fig. 47. But when we make a long stab culture with a strict anaërobic the development occurs only near the bottom of the line of puncture (Fig. 48).

We may then, if we have a pure culture to start with, propagate these anaërobic bacilli in *long stab cultures*. It is best to use tubes which have been recently sterilized, as boiling expels the air from the culture medium; and a very slender needle should be used in making the inoculation. To prevent the absorption of oxygen a layer of sterilized olive oil may be poured into the tube after the inoculating puncture has been made, or it may be filled up with agar jelly which has been cooled to about 40° C. Roux has proposed to prevent the absorption of oxygen by the culture medium by planting an aërobic bacterium—Bacillus subtilis—upon the surface, after making a long stab culture with the anaërobic species. The agar jelly is first boiled and quickly cooled; the inoculation is then made with a slender glass needle; some sterile agar cooled to 40° C. is poured into the tube, and when this is solid the aërobic species is planted upon the surface. The top of the test tube is then closed hermetically and it is placed in the incubating oven. The aërobic species exhausts the oxygen in the upper part of the tube by its growth on the surface of the culture medium, and the anaërobic species grows at the bottom of the tube. To obtain material for a new culture or for microscopical examination the test tube is broken near its bottom.

Cultures in liquid media may be made by exhausting the air in a suitable receptacle or by displacing it with hydrogen gas. The first-mentioned method has been largely used in Pasteur's laboratory, but methods in which hydrogen gas takes the place of atmospheric air in the culture tube are more easily applied and require simpler apparatus. The flask shown in Fig. 49 may be used in connection with an air pump. The sterile culture liquid is first introduced into a long-necked flask and inoculated with the anaërobic bacillus to be cultivated. The neck of the flask is then drawn out in a flame at *c*. The open end is then connected with a Sprengle's pump or some other apparatus for exhausting the air. The flask is placed in a water bath at 40° C., which causes ebullition at the diminished pressure, and the exhaustion is continued for about half an hour. The narrow neck is then sealed at *c* by the use of a blowpipe flame.

The flask shown in Fig. 49, which can be made from a test tube, may also be used in connection with a hydrogen apparatus. In this case a slender glass tube is passed into the flask, as shown in Fig.

50, and this is connected with a hydrogen apparatus by a rubber tube, The hydrogen is allowed to bubble through the culture liquid in a full stream for ten to fifteen minutes, in order that all of the oxygen in the flask may be removed by displacement. Then, while the gas is still flowing, the flask is sealed at *a* with a blowpipe flame, the hydrogen tube being left in position and melted fast to the flask. Some little skill is required in the successful performance of the last step in this procedure, and it will be easier for those who are not skilful in the use of the blowpipe to use Salomonson's tube, shown in Fig. 51. In this, hydrogen is admitted through the arm *b*, and escapes through the cotton plug *a*. The vertical tube is sealed at *c* while the gas is flowing, and then the horizontal tube at *b*.

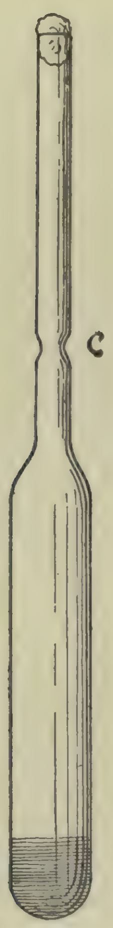

FIG. 49.

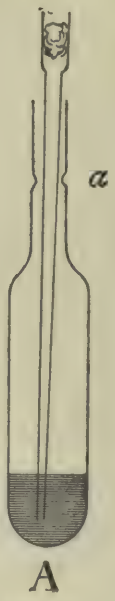

FIG. 50.

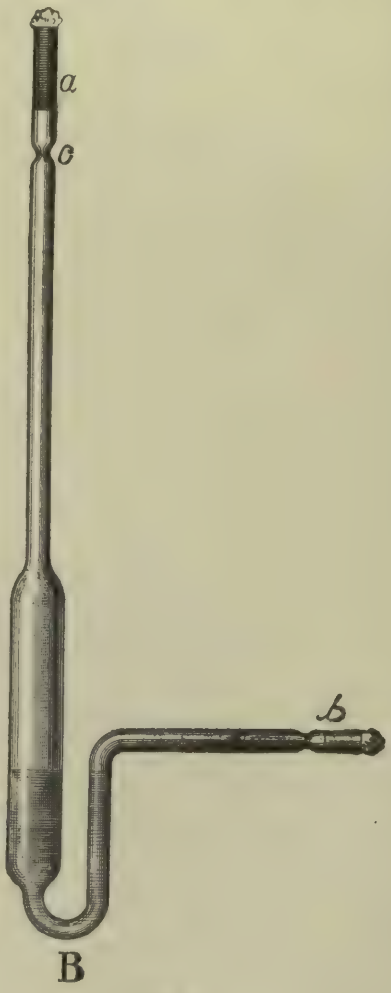

FIG. 51.

Fränkel's Method.—Instead of these tubes specially made for the purpose, an ordinary test tube may be used, as recommended by Fränkel. This is closed by a soft rubber cork through which two glass tubes pass—one, reaching nearly to the bottom of the test tube, for the admission of hydrogen, which passes through the liquefied culture medium ; and the other a short tube for the escape of the gas. The outlet tube is sealed in the flame of a lamp while the gas is freely flowing, and after sufficient time has elapsed to insure the complete expulsion of atmospheric oxygen—which, when the hydrogen flows freely, requires about four minutes (Fränkel)—melted paraffin is applied freely to the rubber stopper to prevent leakage of the hydrogen and entrance of oxygen. A roll tube may then be made after the manner of Esmarch, and, after colonies have developed, the anaërobic culture will appear as shown in Fig. 52.

To isolate anaërobic bacteria in pure cultures it is well to make a series of dilutions as heretofore described for aërobic cultures ; we will then usually obtain isolated colonies in tube No. 2 or No. 3 of a series, and by removing the rubber stopper we may transplant bacteria from these colonies to deep stab cultures in nutrient gelatin or agar.

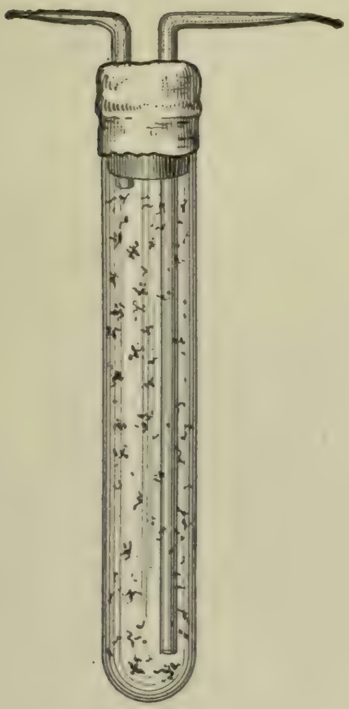
FIG. 52.

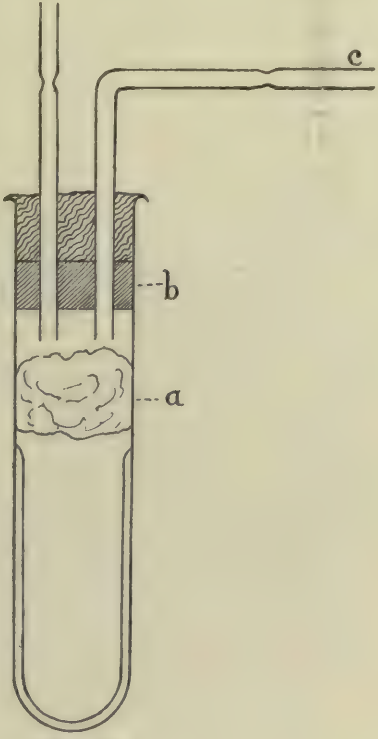

FIG. 53.

The Writer's Method.—The following simple method has been successfully employed by the writer:

Three Esmarch roll tubes are prepared as is usual for aërobic cultures. The cotton air filter, or a portion of it, is then pushed down the tubes for a short distance, as shown at *a*, Fig. 53. A section of a soft rubber stopper carrying two glass tubes is then pushed into the test tube for about half an inch, as shown at *b*, Fig. 53. The space above the cork is then filled with melted sealing wax, which I have found to prevent leakage better than paraffin, which contracts upon cooling. The test tube is inverted while hydrogen is passed through the tube *c*, and by reason of its levity the gas quickly passes through the cotton air filter and displaces the oxygen in the test tube (Fig. 54). After allowing the gas to flow for a few minutes the outlet tube is first sealed in a flame and then the inlet tube. As the cotton filter is interposed between the rubber stopper and the culture material, no special precautions need be taken for the sterilization of the rubber cork and the glass tubes which it carries.

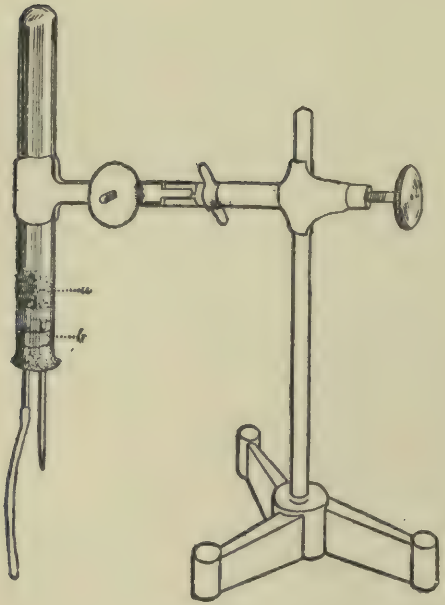

FIG. 54.

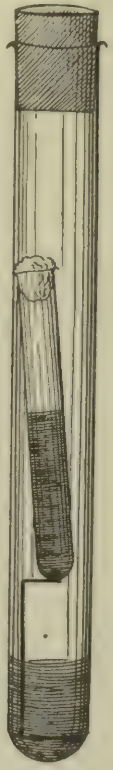

FIG. 55.

This method is more convenient than that previously described, and the only objection to it is that the oxygen is not completely removed from the film of solid gelatin or agar attached to the walls of the test tube. But by passing the hydrogen for a long time it would seem that by diffusion the oxygen remaining in this thin layer would be gotten rid of. At all events, this method will serve for all except the very strict anaërobics.

Method of Esmarch.—The following method has been proposed by Esmarch : Three roll tubes are made in the usual way, and into these liquid gelatin, that is nearly cooled to the point of becoming solid, is poured. This fills the tube without melting the layer of gelatin, previously cooled upon its walls, which contains the bacteria under investigation. When the anaërobic colonies have developed the test tube must be broken to get at them, or the cylinder of gelatin may be removed by first warming the walls of the tube.

Another *method, recommended by Liborius,* consists in distributing the bacteria in test tubes nearly filled with nutrient gelatin or agar which has been recently boiled to expel air. Colonies of anaërobic bacteria will develop near the bottom of such a tube, while the aërobic species will only grow near the surface. The cylinder of jelly is removed by heating the walls of the tube, and sections are made with a sterilized knife for the purpose of obtaining material from individual colonies for further cultures, etc.

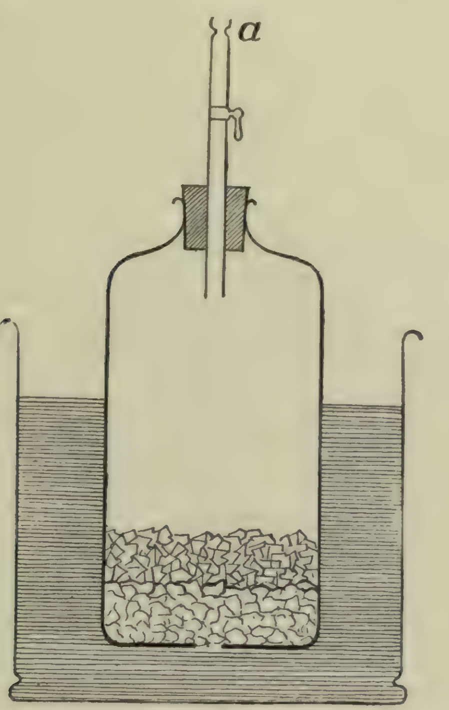

Fig. 56.

Koch and his pupils are in the habit of testing the aërobic character of bacteria in plate cultures by covering the recently made plates with a thin sheet of mica which has been sterilized by heat. The strictly aërobic species do not grow under such a plate ; but, according to Liborius, the exclusion of oxygen is not sufficiently complete for the growth of strict anaërobics.

Buchner's Method consists in the removal of oxygen by means of pyrogallic acid. The anaërobic species under investigation is planted in recently boiled agar jelly in a small test tube. This is placed in a larger tube having a tightly fitting rubber stopper, as shown in Fig. 55. The small tube is supported by a bent-wire stand, and in the lower part of the large tube are placed ten cubic centimetres of a ten-per-cent solution of caustic potash, to which one gramme of pyrogallic acid is added. The absorption of the oxygen takes some time, but, according to Buchner, it is finally so complete that strict anaërobics grow in the small tube.

In practice, cultivation in an atmosphere of hydrogen will be found the most convenient method, and for this any form of hydrogen generator may be used. The writer is in the habit of using the form shown in Fig. 56. A perforation a quarter of an inch in diameter is drilled through the bottom of a wide-mouthed bottle. Some fragments of broken glass are then put into the bottle, forming a layer two or three inches thick. Upon this is placed a quantity of granulated zinc. This bottle has a tightly fitting cork, through which passes a metal tube having a stopcock. The bottle is placed in a glass jar containing diluted sulphuric acid (one part by weight of sulphuric acid to eight parts of water). The acid, rising through the perforation in the bottom of the bottle, when it comes in contact with the zinc gives rise to an abundant evolution of hydrogen, which escapes by the tube *a* when the stopcock is open. When this is closed the gas forces the acid back from contact with the zinc. To remove any trace of oxygen present the gas may be passed through a solution of pyrogallic acid in caustic potash.

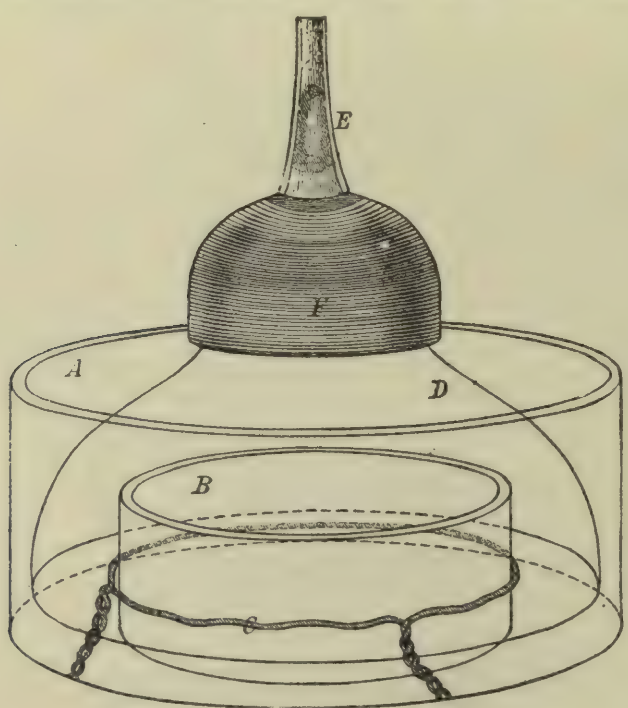

FIG. 57.

Evidently plates prepared by Koch's method, or Esmarch roll tubes, may be placed in a suitable receiver and the air exhausted, or hydrogen substituted for atmospheric air. Such an apparatus for hydrogen has been devised by Blücher and is shown in Fig. 57. A glass dish, A, contains a smaller dish, B, which has a diameter of about seven centimetres. The small dish is kept in its position in the centre of the larger one by the wire ring, having three projecting arms, which is shown in the figure. The culture medium containing the anaërobic bacteria to be cultivated is poured into the small dish and the glass funnel D is put in position. This is held in its place by a weight of lead which encircles the neck of the funnel at F. A mixture of glycerin and water (twenty to twenty-five per cent) is poured into the dish A to serve as a valve to shut off the atmospheric air from the interior of the funnel D. Hydrogen gas is introduced through the tube E, which is connected by a rubber tube with a hydrogen apparatus.

A somewhat similar apparatus has been devised by Botkin, in which the hydrogen is admitted beneath a bell jar covering small glass dishes containing the culture medium. We believe that in practice the writer's method (page 83), in which Esmarch roll tubes are first made, will be found more convenient than either of the last-mentioned methods of preserving plates in an atmosphere of hydrogen ; or roll tubes may be prepared in the way usually practised in cultivating aërobic bacteria, and these may be placed in a suitable receptacle which can be filled with hydrogen.

The addition of a reducing agent to the culture medium favors the growth of anaërobic bacteria. Kitasato and Weil have recommended formic acid or sodium formate, in the proportion of 0.3 to 0.5 per cent. Theobald Smith has found 0.3 to 0.5 per cent of glucose to be a useful addition with the same object in view.

X.

INCUBATING OVENS AND THERMO-REGULATORS.

THE saprophytic bacteria generally, and many of the pathogenic species, grow at the ordinary temperature of occupied apartments (20° to 25° C.); but some pathogenic species can only be cultivated at a higher temperature, and many of those which grow at the "room temperature" develop more rapidly and vigorously when kept in an incubating oven at a temperature of 35° to 38° C. Every bacteriological laboratory should therefore be provided with one or more brood ovens provided with thermo-regulators to maintain a constant temperature. These incubating ovens are made with double walls surrounding an air chamber. The space between the double walls is filled with water, which is usually heated by a small gas flame. The gas passes through the thermo-regulator, and its flow is automatically controlled for any temperature to which this is adjusted. The exterior of the incubating oven is covered with felt or asbestos to prevent the loss of heat by radiation. A simple and cheap form which answers every purpose is shown in Fig. 58. The quadrangular box with double walls should be made of zinc or copper. An outer metal door covered with non-conducting material, and an inner door of glass, give access to the interior space; and a thermometer introduced through an aperture in the top (Fig. 58, *b*) shows the temperature of this space when the door is closed. The stopcock *e* permits the drawing off of the water from the space between the double walls, and the glass tube *d* shows the height of the water, as it is connected with the space containing it. The thermo-regulator passes through an aperture at one side of the oven into the water, the temperature of which controls the flow of gas.

The ordinary thermo-regulator is shown in Fig. 59 as manufactured by Rohrbeck. A glass receptacle, shaped like an ordinary test tube, has an arm, *c*, for the escape of the gas, which enters by the bent tube *a*, which passes through a perforated cork and is adjustable up and down. Tube *a* is connected with the gas supply and tube *c* with the burner by means of rubber tubing. A glass partition extending downward as a tube, *g*, makes an enclosed space in the lower part of the instrument, and this, when immersed in water, acts as a thermometer bulb. This space contains mercury below and air or the vapor of ether above. When the air is expanded by heat the mercury is forced up the tube *g* until it meets the end of the inlet tube for gas at *h*, and by shutting off the flow of gas prevents the temperature from going any higher. A small opening in the inlet tube at *e* permits a small amount of gas to flow, so that the flame under the brood oven (Fig. 58, *f*) may not be entirely extinguished. The lower end of the bent tube *a* is bevelled, so that a triangular opening is formed, which is closed gradually by the rising mercury, instead of abruptly as would be the case if the lower end of the tube *a* were cut off square. To adjust the temperature in the air space of the incubating oven when the thermo-regulator is in position, a full flow of gas is admitted to the burner until the thermometer (Fig. 58, *b*) shows the desired temperature; then the bent tube *a* is pushed down through the cork until its lower extremity meets the mercury and the flame *f* is somewhat reduced. The apparatus is then left for a time, to see whether the flame runs too high or too low, and a further adjustment is made. When the changes in the exterior temperature are slight and the gas pressure regular the temperature in the air chamber is controlled with great precision. But this is not the case under the reverse conditions. Changes in

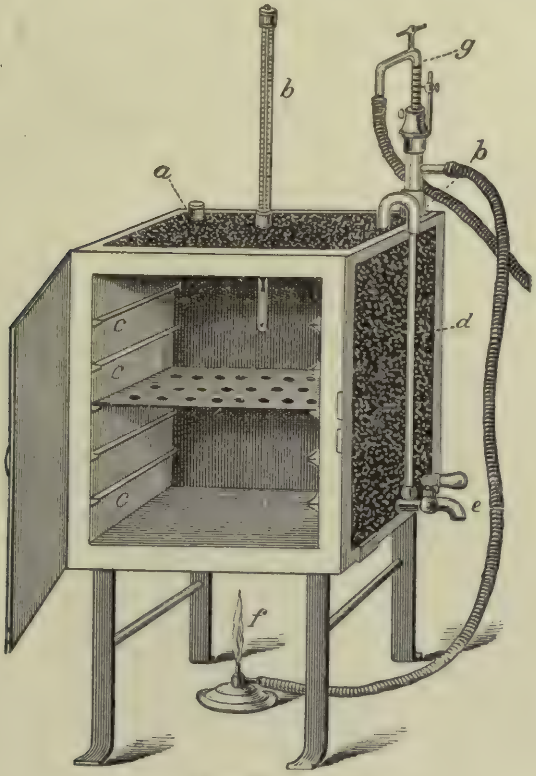

FIG. 58.

the pressure of gas, especially, interfere with the maintenance of a constant temperature, and for this reason a pressure regulator will be required when great precision is desired. That of Moitessier is commonly used in bacteriological laboratories (Fig. 60). But for most purposes variations of temperature of 1° to 2° C. are not of great importance. For ordinary use a brood oven should be regulated to about 35° to 37° C. It is best to have a little cylindrical screen of mica around the gas jet beneath the incubating oven, for the purpose of preventing the flame from being extinguished by currents of air (Fig. 61).

Koch's ingenious automatic device for shutting off the gas if the flame is accidentally extinguished is shown in Fig. 62.

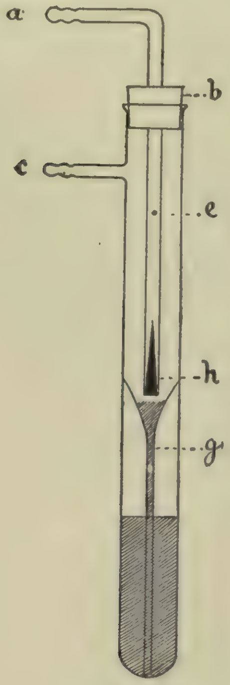

FIG. 59.

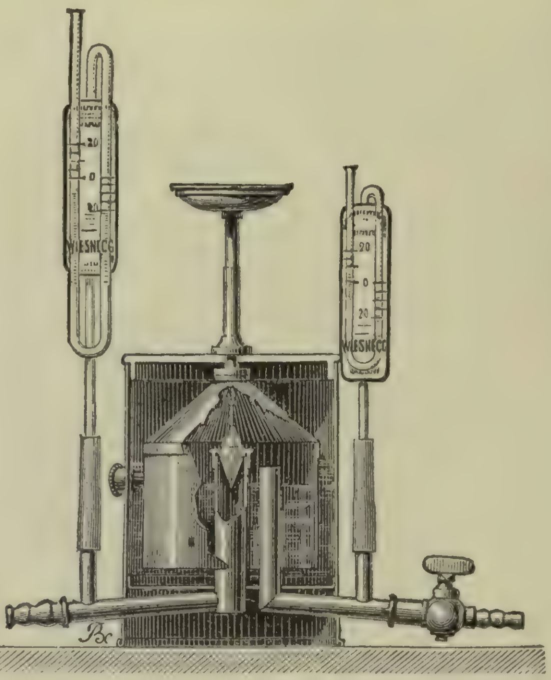

FIG. 60.

Another form of thermo-regulator, which answers very well, is that of Reichert (Fig. 63). In this the gas enters at *a* and escapes at *c*. The mercury, which fills the bulb, shuts off the gas at the point for which the instrument is regulated. By means of the screw *d* the height of the mercury in the tube may be very accurately adjusted for any desired temperature.

The regulator of Bohr, shown in Fig. 64, is more sensitive than that of Reichert, and rather simpler in construction than the usual form shown in Fig. 59. The thermometer bulb *a* contains only air, and the gas which passes through the tube *f* is shut off at the proper temperature by the mercury in the U-shaped tube *c*. The stopcock *b* is left open when the bulb *a* is immersed in the water bath, and when the proper temperature is reached is closed so as to confine the air in the bulb. An increase of temperature now causes the air in the bulb to expand, the mercury in the U-tube is forced up and shuts off the gas flowing through the tube *f* at its lower extremity, *d*. A small opening, *e*, permits sufficient gas to pass to

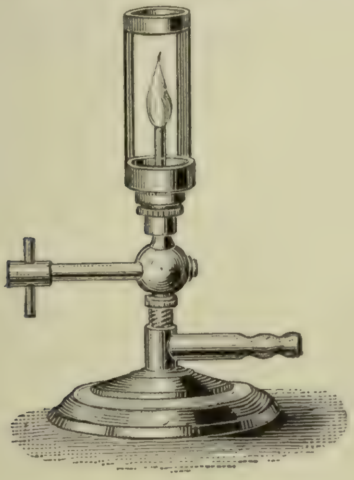

FIG. 61.

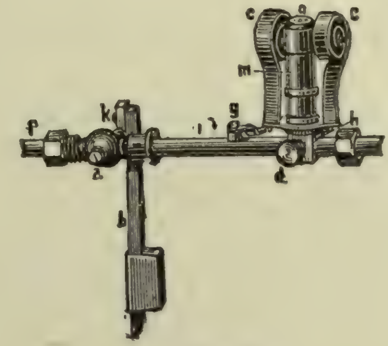

FIG. 62.

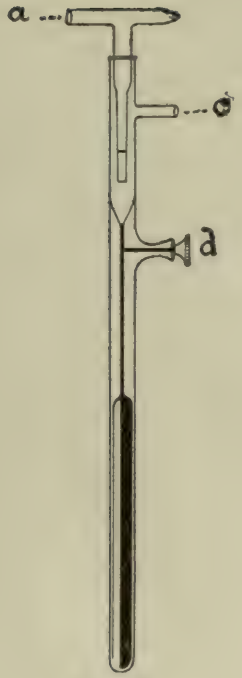

FIG. 63.

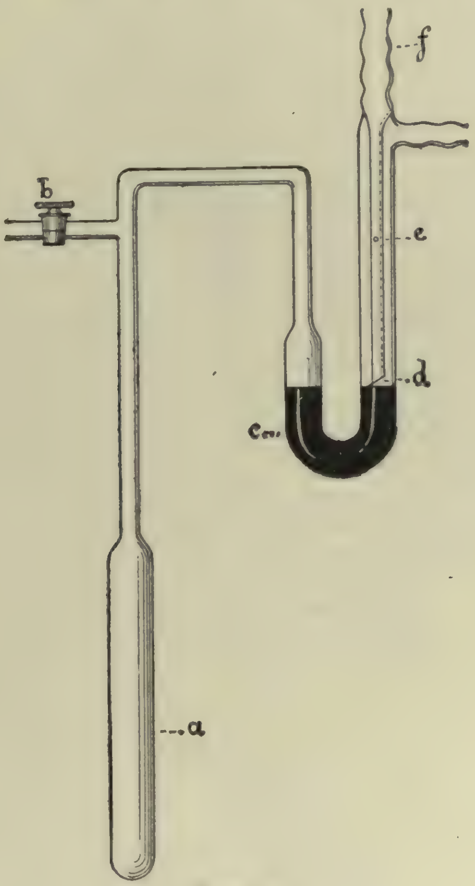

FIG. 64.

maintain a small flame which must not be sufficient by itself to keep up the desired temperature in the water bath.

Altmann has recently (1891) described a thermo-regulator which is made by Müncke, of Berlin, and which is shown in Fig. 65. This is said to act with great precision. It is a modification of Reichert's regulator. Its mode of action will be readily understood by a reference to the figure.

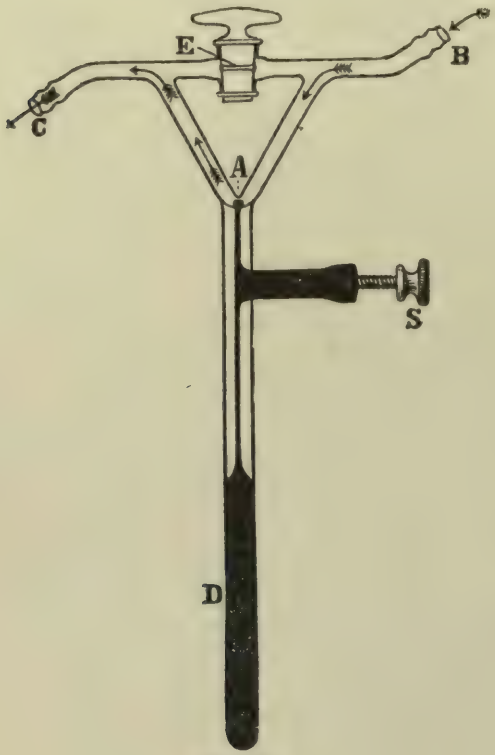

Fig. 65.

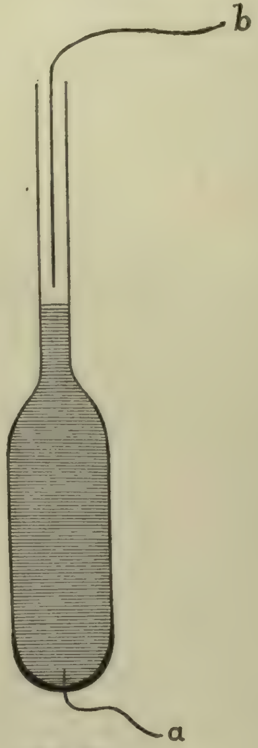

Fig. 66.

A thermo-regulator which gives very accurate results, which are not influenced by differences in pressure, is that invented by the writer over thirty years ago. The regulating thermometer may contain mercury only, or air and mercury, as shown in the thermo-regulator for gas (Fig. 59). In the simplest form a large bulb containing mercury is used, and a platinum wire is hermetically sealed in the glass so as to have contact with the mercury (Fig. 66, *a*).

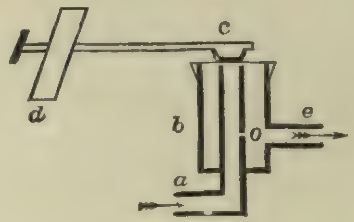

Fig. 67.

Another platinum wire passes down the tube of the thermometer, *b*, and is adjustable for any desired temperature. The gas passes through a valve which is controlled by an electro-magnet. A simple form of valve is shown in Fig. 67. The bent tube *a* is connected with the gas supply by a piece of rubber tubing. The upright arm of this tube is enclosed in a larger tube, *b*, having an outlet, *e*, which is connected with the burner under the incubating oven. The upper end of this larger tube is closed by means of a piece of sheet rubber, which prevents the escape of gas. When this is depressed by means of the lever *c*, the flow of gas through the valve is arrested. The lever *c* has attached to it the armature *d*, and is operated by an electro-magnet under the control of the regulating thermometer.

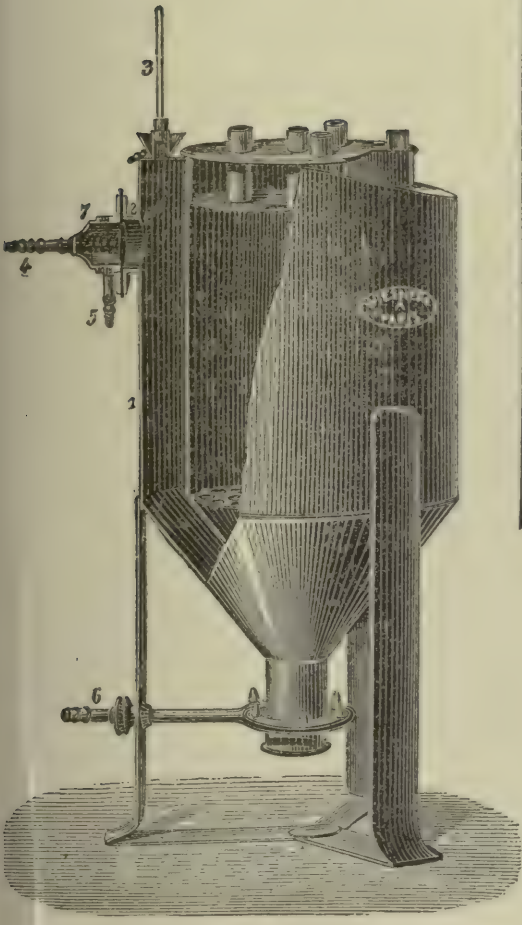

FIG. 68.

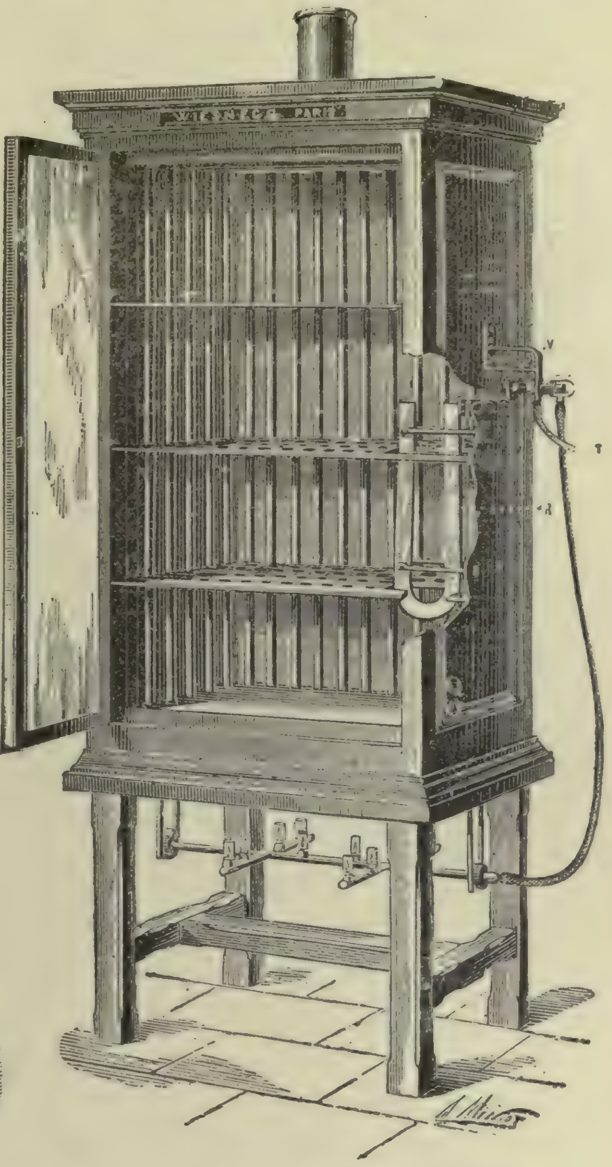

FIG. 69.

When the thermometer is immersed in a water bath the temperature of which it is desired to regulate, and the proper electric connections are made, it acts as a circuit breaker. When the desired temperature is reached the mercury in the tube of the thermometer touches the wire *b* (Fig. 66), an electric circuit is completed, and the valve is closed, shutting off the gas supply and preventing the temperature from going any higher. When contact is broken in the thermometer tube the valve opens and permits the gas to flow again. A small opening, *o* (Fig. 67), permits the constant flow of a sufficient amount of gas to prevent the flame from being extinguished. In practice, however, it is better to have a small side jet of gas, quite independent of that which passes through the valve, which burns constantly and relights the principal jet when the valve is opened. This apparatus is very well adapted for regulating the temperature of a water bath with precision, but for general use in connection with incubating ovens the ordinary gas regulator is preferable, on account of the trouble connected with keeping a galvanic battery in order when it is required to act at frequent intervals "on a closed circuit," for weeks and months together.

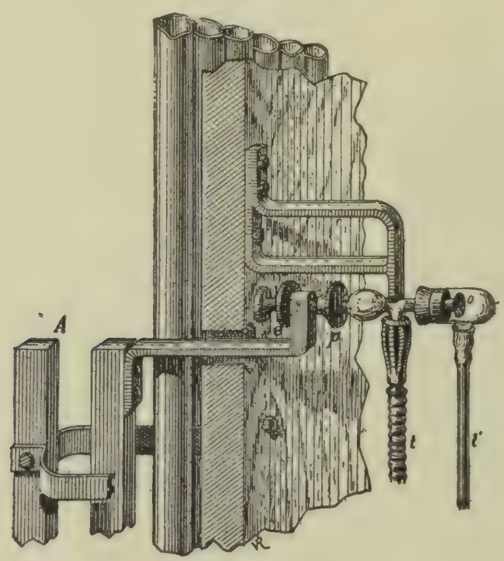

Fig. 70.

The incubating apparatus of D'Arsonval is shown in Fig. 68. It is a cylindrical vessel of copper having double walls, and is provided with the thermo-regulator of D'Arsonval, by which very accurate regulation is maintained at any desired temperature. In its form this apparatus is not as convenient as are the brood ovens made in the form shown in Fig. 58, with a swinging door which gives easy access to the interior, which is provided with one or more shelves upon which the cultures are placed. Various modifications of this simple and convenient incubating oven are manufactured by Rohrbeck and by Müncke, of Berlin. The apparatus of D'Arsonval, and other forms in favor at the French capital, may be obtained from Wiesnegg, of Paris. The last-named manufacturer also supplies the incubating oven and thermo-regulator described by Roux (1891). This is shown in Fig. 69. The regulator is formed of two metallic bars, one of steel and the other of zinc ; these are soldered together in the shape of a letter U ; the regulator is seen in position in the cut (Fig. 69). The most dilatable metal (zinc) is on the outside. When the temperature is raised the arms of the U approach each other, and the reverse when it falls. The method by which regulation is effected is shown in Fig. 70. The U-shaped regulator is placed vertically, and one of its branches, A, is firmly fixed to the wall of the incubating oven ; the other, free arm carries a horizontal bar which projects through the wall of the incubator in an opening which permits it to move freely under the influence of a change in the temperature within. The end of this projecting bar is turned up at a right angle and the screw *p* passes through it ; this can be fixed at any desired point by means of the nut *e*. The end of the screw *p* rests against the stem of a conical brass valve which controls the flow of gas. The valve is closed by a spiral spring and opened by the screw *p* under the control of the thermo-regulator.

In the absence of gas incubating ovens may be heated by a small petroleum lamp, and various devices have been invented for controlling the temperature. Reichenbach describes an apparatus for this purpose in the *Centralblatt für Bakteriologie*, Vol. XV., p. 847, 1894. Dr. Borden of the U. S. Army has also invented a thermo-regulator to be used in connection with a petroleum lamp. In the absence of any regulating apparatus an incubating oven may be kept at a tolerably uniform temperature by personal supervision—adjusting the flame of the lamp and its distance from the bottom of the oven according to the changes in the external temperature. For most bacteria a variation of several degrees is not important, so long as the temperature is not allowed to rise above 37° to 38° C. The typhoid bacillus, the diphtheria bacillus, the anthrax bacillus, the pus cocci, and most saprophytic bacteria grow at the ordinary room temperature, and may therefore be cultivated without any form of incubating oven or thermo-regulator.

XI.

EXPERIMENTS UPON ANIMALS.

THE pathogenic power of various bacteria has been demonstrated by injecting pure cultures into susceptible animals. As a rule, the herbivora are more susceptible than the carnivora, and this is perhaps to be explained in accordance with the theory of natural selection. Carnivorous animals often feed upon the bodies of animals which have succumbed to infectious diseases, and upon dead animals in which putrefactive changes have commenced. In their struggles with each other they are wounded by teeth and claws soiled with infectious material which would cause a fatal disease if inoculated into the more susceptible herbivorous animals. As this has been going on for ages, we may suppose that, by survival of the fittest, a race tolerance has been acquired. The lower animals have their own infectious diseases, some of which are peculiar to certain species and some common to several. As a rule, the specific infectious diseases of man cannot be transmitted to lower animals, and man is not subject to the diseases of the same class which prevail among animals. But certain diseases furnish an exception to this general rule. Thus tuberculosis is common to man and several of the lower animals; relapsing fever may by inoculation be transmitted to monkeys; diphtheria may be transmitted to pigeons and guinea-pigs. On the other hand, anthrax and glanders may be contracted by man as a result of accidental inoculation or contact with an infected animal.

Nearly allied species sometimes present very remarkable differences as to susceptibility. Thus the bacillus of mouse septicæmia is fatal to house mice but not to field mice, while, on the other hand, field mice are killed by the bacillus of glanders and house mice are immune from this pathogenic bacillus.

The animals most commonly used for testing the pathogenic power of bacteria are the mouse, the guinea-pig, and the rabbit. Domestic fowls and pigeons are also useful for certain experiments. The dog and the rat are of comparatively little use on account of their slight susceptibility.

Inoculations are made directly into the circulation through a vein, into the subcutaneous connective tissue, or into one of the serous cavities—usually the peritoneal.

The ordinary hypodermic syringe may be used in making injections, but this is difficult to sterilize on account of the leather piston, and complications are liable to arise from its use which it is best to avoid. The best way to sterilize a piston syringe is to wash it thoroughly with a solution of bichloride of mercury of 1 : 1,000, and then to remove every trace of bichloride by washing in alcohol. But one never feels quite sure that the most careful washing will insure sterilization, and it is best to use a syringe which may be sterilized by heat, such as that of Koch, shown in Fig. 71. In this the metal point and glass tube are easily sterilized in a hot-air oven. Fluid is drawn into the syringe and forced out of it by a rubber ball which has a perforation to be covered by the finger.

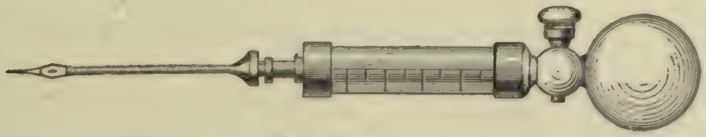

FIG. 71.

The writer has for some years been in the habit of making injections in animals with an improvised glass syringe. This is made from a piece of glass tubing in the same form as the collecting tubes heretofore described. A bulb is blown at one end of the tube, and the other end is drawn out to form a slender tube which serves as the needle of the syringe (Fig. 72). By gently heating the bulb in an alcohol lamp and immersing the open end of the capillary tube in the fluid to be injected, this rises into the syringe as the expanded air cools. Having introduced the glass point beneath the skin or into the cavity of the abdomen of the animal to be injected, the contents of the tube are forced out by again heating the bulb by means of a small alcohol lamp. The glass point is easily forced through the thin skin of a mouse or of a young rabbit; but for animals with a thicker skin it is necessary to cut through, or nearly through, the skin with some other instrument. A small pair of curved scissors answers very well for this purpose.

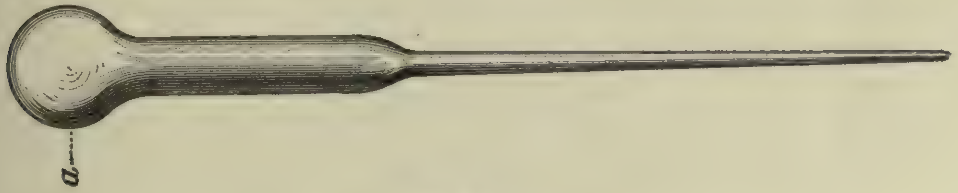

FIG. 72.

Generally, in making injections into animals, it is customary to remove the hair for some distance around the point of inoculation with scissors and razor, and then to sterilize the surface by careful washing with a solution of bichloride of mercury. This precaution is necessary in researches in which pathogenic bacteria are being tested, in order to remove any possibility of accidental inoculation with germs other than those under investigation, and, as a consequence, a mistaken inference as to the pathogenic action of the species under investigation. But when we know the specific pathogenic power of a certain microörganism it is hardly necessary to take this precaution, as a few drops of culture will contain millions of the bacteria, while contamination, if it occurs from the surface of the body, must be by a comparatively small number of bacteria, which are likely to be of a harmless kind which will have no influence on the result of the experiment.

Instead of sterilizing the surface, the writer usually clips away a small portion of skin with curved scissors, not cutting deep enough to draw blood, but leaving a bare surface through which the point of the syringe can be introduced with very little danger of carrying bacteria into the connective tissue other than those contained in the syringe.

In making injections into the peritoneal cavity care must be taken not to wound the liver or the distended stomach. The intestine is not very likely to be wounded, as it slips out of the way. By seizing a longitudinal fold of the abdominal wall and pushing the point of the syringe quite through it, and then releasing the fold and carefully withdrawing the instrument until the point remains in the cavity, the danger of wounding the intestine will be reduced to a minimum.

Injections into the circulation are made by exposing a vein and carefully introducing the needle of the syringe in the direction of the blood current. Care must of course be taken not to inject air. In the rabbit one of the large veins of the ear may be conveniently penetrated by the point of a hypodermic syringe without any previous dissection. The ear is first washed with a solution of bichloride of mercury or simply with warm water. The animal had better be carefully wrapped in a towel to control its movements. The veins are distended by compressing them near the base of the ear. When the point of the needle has not been properly introduced, and the fluid to be injected escapes in the surrounding connective tissue, it will commonly be best to withdraw the syringe and make the attempt upon another vein. As pointed out by Abbott, the needle of the syringe should be ground flat at the point, and not curved as is commonly the case.

Large quantities of fluid may be injected into the cavity of the abdomen or into the circulation by slowly forcing the fluid through a slender canula, properly introduced, which is coupled with a large syringe by means of rubber tubing, or with a glass receptacle from which the fluid is forced by the pressure of air pumped in with a rubber hand ball.

Mice are usually injected subcutaneously near the tail. The little animal is first seized by a long pair of forceps, or "mouse tongs," and the hair is clipped away on the back just above the tail. If solid material is to be introduced a little pocket is made with scissors or with a lancet, into which the infectious material is carried by means of a platinum needle or slender forceps. Liquids may be injected by the little glass syringe heretofore described, the point of which is easily forced through the skin.

Pasteur's method of inoculating rabbits with the virus of hydrophobia consists in trephining the skull and injecting the material beneath the dura mater. An incision through the skin is first made to one side of the median line a short distance back of the eyes. The edges of the wound are separated, and a small trephine (five or six millimetres in diameter) is used to remove a button of bone. The emulsion of spinal cord from a hydrophobic animal is then carefully injected beneath the dura mater—two or three drops will be sufficient. The wound is washed out with a two-per-cent solution of carbolic acid and closed with a couple of sutures.

Injections into the intestine are made by carefully opening the abdomen with antiseptic precautions, gently seizing a loop of the intestine, and passing the point of the syringe through its walls ; the loop is then returned and the incision in the walls of the abdomen carefully closed with sutures and dressed antiseptically.

Inoculations into the anterior chamber of the eye of rabbits and other animals have frequently been practised, and offer certain advantages in the study of the local effects of pathogenic microörganisms. The animal should be fastened to an operating board, belly down, and its head held by an assistant, who at the same time holds the eyelids apart. The conjunctiva is seized with forceps to steady the eye, and an incision about two millimetres long is made through the cornea with a cataract knife. Through this opening a small quantity of a liquid culture may be injected, or a bit of solid material introduced with slender curved forceps.

Ordinary injections give but little pain and do not call for the use of an anæsthetic. When anæsthesia is required ether will usually be preferable to chloroform. Rabbits, especially, are very apt to die from chloroform, no matter how carefully it may be administered. Dogs, rats, and mice stand ether very well. The smaller animals may be brought under the anæsthetic by placing them in a covered jar into which a pledget of cotton wet with ether has been dropped. Before making injections into the anterior chamber of the eye it is well to use a two-per-cent solution of cocaine as a local anæsthetic.

Mice which have been inoculated are usually kept in a glass jar having a wire-gauze cover. A quantity of cotton is put into the jar to serve as a shelter for the little animal, and it is well to partly fill the jar with dry sawdust. Larger animals are kept in suitable cages of wire or wood, and, as a rule, each one should be kept in a separate cage while under observation after an inoculation experiment.

In experimenting upon animals the following points should be kept in view and noted :

(*a*) *The age and weight of the animal.* Young animals are, as a rule, more susceptible than older ones, and with many pathogenic bacteria the lethal dose of a culture bears some relation to the size of the animal.

(*b*) *The point of inoculation.* Injections into the circulation are generally more promptly fatal and require a smaller dose than those into a serous cavity or into the connective tissue. Pathogenic bacteria introduced into the abdominal cavity reach the circulation more promptly than those injected subcutaneously. But certain microörganisms owe their pathogenic power to the local effect about the point of inoculation and the absorption of toxic products formed in the limited area invaded, and do not enter the general circulation, or at least do not multiply in the circulating fluid, and quickly disappear from it.

(*b*) *The age of the culture injected.* Old cultures sometimes have greater and sometimes less pathogenic potency than recent cultures. Some kinds of virus become "attenuated" when kept. But when the pathogenic power depends chiefly upon toxic products formed during the growth of the bacteria, old cultures are, as a rule, more potent than those recently made.

(*d*) *The medium in which the pathogenic bacteria are suspended.* Cultures in albuminous media, like blood serum, are in some cases more potent than bouillon cultures ; and the virulence of several pathogenic bacteria is greatly intensified by successive cultures—by inoculation—in the bodies of susceptible animals. Ogston found that pus cocci cultivated in the interior of eggs had an increased virulence. According to Arloing, Cornevin, and Thomas, the activity of a culture of the bacillus of symptomatic anthrax is doubled by adding one-five-hundredth part of lactic acid to the culture fluid.

(*e*) *The quantity injected* is evidently an essential point when the result depends largely upon the toxic products formed in the culture medium. It is also an essential point when pathogenic bacteria are injected which kill susceptible animals in very minute doses, for it has been shown by the experiments of Watson Cheyne and others that in the case of some of these, at least, there is a limit below which infection does not occur.

Inoculated animals should be carefully observed, and a note made of every symptom indicating a departure from the usual condition of health, such as fever, loss of activity, loss of appetite, weakness, emaciation, diarrhœa, convulsions, dilated pupils, the formation of an abscess or a diffuse cellulitis extending from the point of inoculation, etc. The temperature is usually taken in the rectum. The temperature of small animals, like rabbits and guinea-pigs, varies considerably as a result of external conditions. In the rabbit the normal temperature may be given as about 102° to 103° F. ; in the guinea-pig it is a little lower.

In making a post-mortem examination of an inoculated animal it is best to stretch it out on a board, belly up, by tying its legs to nails or screws fastened in the margin of the board. When the abdomen is dirty, as is usually the case, it should be carefully washed with a disinfecting solution. An incision through the skin is then made in the median line the full length of the body, and the skin is dissected back so as to expose the anterior walls of the abdomen and thorax. These cavities are then carefully opened with a sterilized knife or scissors, and the various organs and viscera examined. Attention should also be given to the appearances at the point of inoculation. To ascertain whether the microörganism injected has invaded the blood, smear preparations should be made with blood obtained from a vein or from one of the cavities of the heart. It will be well also to make a smear preparation from a cut surface of the liver and spleen. In the various forms of acute septicæmia the spleen is usually found to be enlarged. If but few microörganisms are present in the blood and tissues they may escape observation in stained smear preparations, and it will be necessary to make cultures to demonstrate their presence. A little blood from a vein or from one of the cavities of the heart is transferred, by means of a platinum loop (*öse*) or a sterilized collecting tube (see page 38), to a test tube containing liquefied nutrient gelatin or agar-agar, and an Esmarch roll tube is made. This is put aside for the development of colonies from any scattered bacteria which may be present. As a rule, it will be best to make agar cultures, as these can be placed in the incubating oven at 35° to 38° C. Stab cultures may also be made and will serve to show the presence of microörganisms, but will not give information as to how numerous they may be. The roll tube also has the advantage of showing whether there is a mixed infection or whether a pure culture of a single microörganism is obtained from the blood. In the same way cultures may be made from material obtained from the liver or spleen, and it may happen that one or both of these organs contain bacteria when none are found in the blood. Before passing the platinum needle or collecting tube into the organ, the surface, which has been more or less exposed to contamination, should be sterilized by applying to it a hot spatula; then at the moment of lifting the spatula the sterilized needle is introduced into the interior of the organ, and the blood and crushed tissue adhering to it at once carried over to the culture medium. Or blood obtained with proper precautions from a vein, a cavity of the heart, or the interior of the spleen or liver, may be used to inoculate another animal.

Animals are also sometimes inoculated by excoriating the cutis as in vaccination. They may also, in rare cases, be infected by introducing cultures into the stomach, either mixed with the food ingested or by injection through a tube. Infection by inhalation is accomplished by causing the animal to respire an atmosphere, in a properly enclosed space, in which the pathogenic organism is suspended, by the use of a spray apparatus for liquid cultures, or some form of powder blower for powders containing the bacteria in a desiccated condition.

One method of obtaining a pure culture of pathogenic bacteria consists in the inoculation of susceptible animals with material containing a pathogenic species in association with others which are not. When the blood is invaded by the pathogenic species and the animal dies from an acute septicæmia, we may usually obtain a pure culture by inoculating a suitable culture medium with a minute drop of blood taken from a vein or from one of the cavities of the heart. Sometimes, however, a mixed infection occurs and some other microörganism is associated in the blood with that one which was the immediate cause of the death of the animal.

XII.

PHOTOGRAPHING BACTERIA.

WELL-MADE photomicrographs are unquestionably superior to drawings made by hand as a permanent record of morphological characters. This being the case, bacteriologists would no doubt resort to this method more generally but for the technical difficulties and the time and patience required in overcoming these. Koch, in his earlier studies, gave much time to photographing bacteria, and with very remarkable success. In his work on "Traumatic Infective Diseases" (1878) he says:

"With respect to the illustrations accompanying this work, I must here make a remark. In a former paper[1] on the examination and photographing of bacteria I expressed the wish that observers would photograph pathogenic bacteria in order that their representations of them might be as true to nature as possible. I thus felt bound to photograph the bacteria discovered in the animal tissues in traumatic infective diseases, and I have not spared trouble in the attempt. The smallest, and in fact the most interesting bacteria, however, can only be made visible in animal tissues by staining them and by thus gaining the advantage of color. But in this case the photographer has to deal with the same difficulties as are experienced in photographing colored objects—*e.g.*, colored tapestry. These have, as is well known, been overcome by the use of colored collodion. This led me to use the same method for photographing stained bacteria, and I have, in fact, succeeded, by the use of eosin-collodion, and by shutting off portions of the spectrum by colored glasses, in obtaining photographs of bacteria which had been stained with blue and red aniline dyes. Nevertheless, from the long exposure required and the unavoidable vibrations of the apparatus, the picture does not have sharpness of outline sufficient to enable it to be of use as a substitute for a drawing, or, indeed, even as evidence of what one sees. For the present, therefore, I must abstain from publishing photographic representations; but I hope, at a subsequent period when improved methods allow a shorter exposure, to be able to remedy this defect."

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[1] The paper referred to is published in Cohn's "Beiträge zur Biologie d. Pflanzen."

Since the above was written considerable progress has been made in removing the technical difficulties, and many bacteriologists have succeeded in making very satisfactory photomicrographs. As specimens of what may be done with the best apparatus and the highest degree of skill, we may call attention to the photomicrographs in the *Atlas der Bakterienkunde* of Fränkel and Pfeiffer, and those of Roux in the *Annales of the Pasteur Institute.* The writer, also, has devoted much time to making photomicrographs which have served as illustrations for several of his published works.

Those who have had no practical experience in making photomicrographs are apt to expect too much and to underestimate the technical difficulties. Objects which under the microscope give a beautiful picture, which we desire to reproduce by photography, may be entirely unsuited for the purpose. In photographing with high powers it is necessary that the objects to be photographed be in a single plane and not crowded together or overlying each other. For this reason photographing bacteria in sections presents special difficulties, and satisfactory results can only be obtained when the sections are extremely thin and the bacteria well stained. Even with the best preparations of this kind much care must be taken in selecting a field for photography. It must be remembered that the expert microscopist, in examining a section with high powers, has his finger on the fine adjustment screw and focuses up and down to bring different planes into view. He is in the habit of fixing his attention on that part of the field which is in the focus and disregarding the rest. But in a photograph the part of the field not in focus appears in a prominent way which mars the beauty of the picture. In a cover-glass preparation made from a pure culture, when the bacteria are well distributed, this difficulty does not present itself, as the bacteria are all lying in a single plane; but the portion of the field which can be shown at one time is limited by the spherical aberration of the objective, which the makers do not seem able to overcome in high-power lenses of wide angle, at least not without loss of defining power.

Usually preparations of bacteria are stained for photography, but with some of the larger forms, such as the anthrax bacillus, very satisfactory photomicrographs may be made from unstained preparations. In this case a small quantity of a recent culture is put upon a slide, covered with a thin cover glass, and placed at once upon the stage of the microscope. The main difficulty to be encountered results from the change of location of the suspended bacteria resulting from the pressure of the objective in focussing. Motile bacteria, of course, cannot be photographed in this way without first arresting their movements by means of some germicidal agent;

and in general it will be found more satisfactory to fix the microörganisms to be photographed to a slide or cover glass by desiccation and heat, and to stain them with one of the aniline colors.

Objects which are opaque cannot be photographed by transmitted light, and objects which have a deep orange or red color are practically opaque for the actinic rays which are at the violet end of the spectrum. Such objects simply intercept the light, but this gives the outlines, and, where there are no details of structure, is all that is required to illustrate the form and mode of grouping. Softer and more satisfactory photomicrographs of bacteria are made when the staining is not such as to entirely arrest the actinic rays. Among the aniline colors Bismarck brown and vesuvin are the most suitable, care being taken, with the larger bacteria especially, not to make the staining too intense. Objects which are transparent for the actinic rays, or nearly so, give a very feeble photographic image, or none at all, on account of the want of contrast in the impression made upon the sensitive plate. This is the case when we attempt to photograph, by ordinary white light, objects which are stained violet or blue. But this want of contrast in the negative can be overcome by the use of specially prepared plates and colored screens of glass interposed between the object and the source of light. The so-called orthochromatic plates are more sensitive to the rays toward the red end of the spectrum than ordinary plates. They are prepared by treating the plates with a solution of eosin, of erythrosin, or of rose bengal (Vogel), and may now be purchased in this country from dealers in dry plates. If we shut off the violet rays by the use of a yellow screen, objects having a yellow or orange color may be photographed upon orthochromatic plates, although the time of exposure will be quite long owing to the comparatively feeble actinic power of the yellow rays.

We may also make photomicrographs of objects stained with methylene blue or with fuchsin, because objects stained with these colors are opaque for the rays from the red end of the spectrum, and sufficiently so with yellow light to give a good photographic contrast. Fränkel and Pfeiffer recommend the use of a green light-filter (green glass screen) for all preparations stained with methyl violet, fuchsin, or methylene blue; and for brown-stained preparations a pure blue light. The writer has been in the habit of using a yellow glass screen for fuchsin-stained preparations, and has had excellent results, but the time of exposure is necessarily long. A yellow glass screen may be prepared by dissolving tropæolin in negative varnish, and pouring this upon a clean glass slide, where it is permitted to dry.

To show bacteria in photographs in a satisfactory manner we require an *amplification* of five hundred to one thousand diameters; and as it is often desirable to make comparisons as to the dimensions of microörganisms which resemble each other in form, it is best to adopt a standard amplification. The writer has himself adopted, and would recommend to others, a standard amplification of one thousand diameters. This is about as high a magnifying power as we can get with satisfactory definition, or as we require, and it is a convenient number when measurements are made from the photograph. The beginner, after having put his apparatus in position, should focus the lines of a stage micrometer upon the screen with the optical apparatus which he proposes to use; then by moving the screen forward or back as required, and carefully focussing the lines, he will ascertain what is the position of the screen for exactly one thousand diameters. If the stage micrometer is ruled with lines which are one one-thousandth of an inch apart, it is evident that when projected upon the screen they should be one inch apart to make the amplification one thousand diameters. But it must be remembered that any change in the position of the optical combination will change the amplification. If, therefore, the cover correction of the objective is changed, or the position of the eyepiece —if one is used—it will be necessary to again adjust the distance of the screen.

Apparatus required.—A first-class immersion objective of one-twelfth of an inch or higher power, a substantial stand which can be placed in a horizontal position, and a camera which can be coupled with the microscope tube, are the essential pieces of apparatus. If sunlight is to be used a heliostat will also be required.

The oil-immersion objectives of any good maker may be used, but the apochromatic objectives and projection eyepieces of Carl Zeiss, of Jena, are especially to be recommended. Indeed, those who can afford it will do well to get Zeiss' complete apparatus, which includes a stand having a mechanical stage and a camera mounted upon a metal frame conveniently provided with focussing appliances, etc. However, good work may be done with less expensive apparatus.

The stand should be substantial and well made, with a delicate, fine adjustment. A mechanical stage is not essential, but is a great convenience in finding and adjusting to the centre of the screen a satisfactory field to photograph. The substage should be provided with a good apochromatic condenser, and with appliances for moving the condensing lens forward and back and for centring it, with diaphragms, etc.

By the use of a high-power objective, like the one-eighteenth-inch oil-immersion of Zeiss, the desired amplification may be obtained without the use of an eyepiece ; and, as a rule, it is best not to use an ordinary eyepiece to secure increased amplification, as this is obtained at the expense of definition. But an amplifier may be used in the tube of the microscope, as first recommended by Woodward. In this case the amplifier must be carefully adjusted with reference to the distance of the screen, to secure the best possible definition.

The projection eyepieces of Zeiss are constructed especially for photography and possess a decided advantage. By the use of his three-millimetre apochromatic oil-immersion objective and projection eyepiece No. 3 we may obtain an amplification of one thousand diameters with excellent definition.

Light.—Sunlight is in many respects the most satisfactory for photography, but has the disadvantage that it is not always available. In some sections of the country weeks may pass without a single clear day suitable for making photomicrographs. In addition to the uncertainty arising from cloudy weather, we have to contend with the fact that the sun is only available for use with a heliostat for a limited time during each day, and that this time is greatly restricted in Northern latitudes during the winter months. When sunlight is to be employed the microscope and camera must be set up in a room having a southern exposure on a line corresponding with the true meridian of the place. The heliostat is placed outside the window in such a position that when properly adjusted the light of the sun will fall upon the condenser attached to the substage of the microscope. The condenser must be carefully centred, so that the circle of light falling upon the screen shall be uniform in intensity and outline.

The calcium, magnesium, or electric light may be used as a substitute for sunlight, but they are all rather expensive, unless, in the case of the electric light, a suitable current is available without the expense of generating it for the special purpose in view. The writer has obtained very good results with the calcium light, but has no experience in the use of the electric light. Woodward, as a result of extended experiments, arrived at the conclusion that "the electric light is by far the best of all artificial lights for the production of photomicrographs." He used a Grove battery of fifty elements to generate the current, and a Duboscq lamp. The current from a dynamo would no doubt be much cheaper and more conveniently used, if an electric-lighting plant was in the vicinity.

The apparatus shown in Fig. 73 was designed by Mr. Pringle for the use of the calcium light. It will serve to illustrate the arrangement of the microscope and camera in connection with any other light as well. An oil lamp may be placed in the position of the oxyhydrogen burner ; or, if sunlight is to be employed, a heliostat will be placed in the same position.

When a colored screen is used this may be placed either before or behind the condensing lens—we prefer to place it behind, although

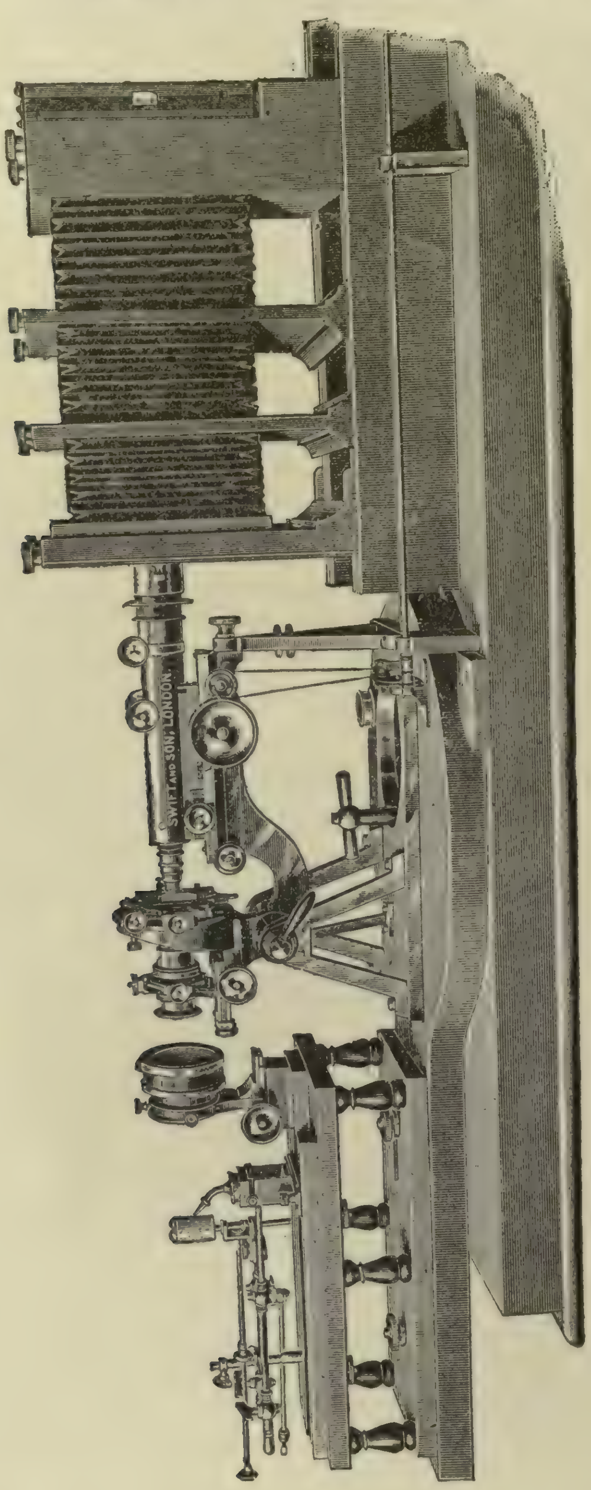

Fig. 73.

Neuhauss has shown that it makes no difference in the length of the exposure.

We cannot in the present volume give full details with reference to the technique of making photomicrographs, but append an account of a form of apparatus which we have used with great satisfaction :

"*Photomicrography by Gaslight.*—Those who have had much experience in making photomicrographs will agree with me that one of the most essential elements of success is the use of a suitable source of illumination.

"Without question the direct light of the sun, reflected in a right line by the mirror of a heliostat, is the most economical and, in some respects, the most satisfactory light that can be used. But we cannot command this light at all times and places, and it often happens that, when we are ready to devote a day to making photomicrographs, the sun is obscured by clouds or the atmosphere is hazy. Indeed, in some latitudes and at certain seasons of the year a suitable day for the purpose is extremely rare. The use of sunlight also requires a room having a southern exposure and elevated above all surrounding buildings or other objects by which the direct rays of the sun would be intercepted. For these reasons a satisfactory artificial light is extremely desirable.

"The oxyhydrogen lime light, the magnesium light, and the electric arc light have all been employed as a substitute for the light of the sun, and all give satisfactory results. I have myself made rather extensive use of the 'lime light,' and think it the best substitute for solar light with which I am familiar. But to use it continuously, day after day, is attended with considerable expense, and the frequent renewal of the supply of gas which it calls for is an inconvenience which one would gladly dispense with.

"These considerations have led some microscopists to use an oil lamp as the source of illumination, and very satisfactory photomicrographs with comparatively high power have been made with this cheap and convenient light. But in my experience the best illumination which I have been able to secure with an oil lamp has called for very long exposures when working with high powers, and, as most of my photomicrographs of bacteria are made with an amplification of one thousand diameters, I require a more powerful illumination than I have been able to secure in this way. And especially so because of the fact that a colored screen must be interposed, which shuts off a large portion of the actinic rays, on account of the staining agent usually employed in making my mounts. The most satisfactory staining agents for the bacteria are an aqueous solution of fuchsin, or of methylene blue, or of gentian violet; and all of these colors are so nearly transparent for the actinic rays at the violet end of the spectrum that a satisfactory photographic contrast cannot be obtained unless we shut off these rays by a colored screen.

"I am in the habit of using a yellow screen for my preparations stained with fuchsin or methylene blue, and have obtained very satisfactory results with the orthochromatic plates manufactured by Carbutt, of Philadelphia, and a glass screen coated with a solution of tropæolin dissolved in gelatin.

"But with such a screen, which shuts off a large portion of the actinic light and increases the time of exposure three- or fourfold, the use of an oil lamp becomes impracticable with high powers, on account of the feebleness of the illumination.

"These considerations have led me to experiment with gaslight, and the simple form of apparatus which I am about to describe is the result of these experiments. I have now had the apparatus in use for several months, during which time I have made a large number of very satisfactory photomicrographs of bacteria from fuchsin-stained preparations with an amplification of one thousand diameters. My photographs have been made with the three-millimetre oil-immersion apochromatic objective of Zeiss and his projection eyepiece No. 3. I use a large Powell and Lealand stand, upon the substage of which I have fitted an Abbe condenser. The arrangement of the apparatus will be readily understood by reference to the accompanying figure.

"A is the camera, which has a pyramidal bellows front supported by the heavy block of wood B; this can be pushed back upon the baseboard which supports it, so as to allow the operator to place his eye at the eyepiece of the microscope. When it is brought forward an aperture of the proper size admits the outer extremity of the eyepiece and shuts off all light except that coming through the objective. C is the microscope, and D the Abbe condenser, supported upon the substage. E is a thick asbestos screen for protecting the microscope from the heat given off by the battery of gas burners F. This asbestos screen has an aperture of proper dimensions to admit the light to the condenser D. The gas burners are arranged in a series, with the flat portion of the flame facing the aperture in the asbestos screen E. The concave metallic mirror G is properly placed to reflect the light in the desired direction. I have not found any advantage in the use of a condensing lens other than the Abbe condenser upon the substage of the microscope. The focussing is accomplished by means of the rod I, which carries at one extremity a grooved wheel, H, which is connected with the fine adjustment screw of the microscope by means of a cord.

"The focussing wheel J may be slipped along the rod I to any desired position, and is retained in place by a set screw. The rod I is supported above the camera by arms depending from the ceiling, or by upright arms attached to the baseboard.

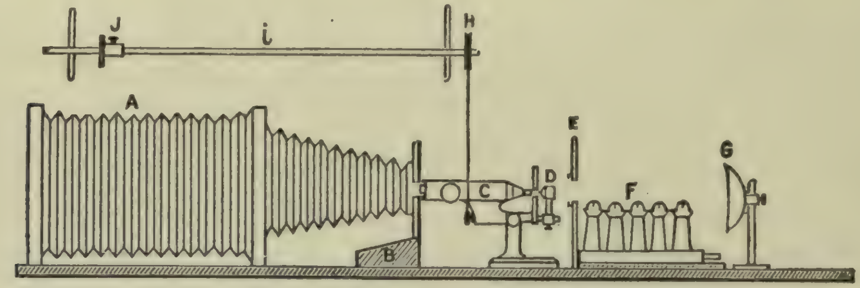

FIG. 74.

"I have lost many plates from a derangement of the focal adjustment resulting from vibrations caused by the passing of loaded wagons in the street adjoining the laboratory in which I work. This has been overcome to a great degree by placing soft rubber cushions under the whole apparatus."[1]

I have recently (1895) seen a gaslight which I believe would prove to be a valuable substitute for ordinary street gas, and I judge that, owing to its superior brilliancy, a single jet would suffice to replace the five burners in a linear series which are shown in the above figure. The gas referred to is acetylene, which may now be obtained in a liquid form in strong metal cylinders. Reference has already been made to the use of an oil light, and for low powers an ordinary lamp with a flat wick may be used. That bacteria may be successfully photographed, with an amplification of one thousand diameters, by means of an oil lamp is shown by the beautiful photomicrographs made by Capt. W. C. Borden, Assistant Surgeon U. S. Army. At my request Dr. Borden has prepared the following detailed account of his method:

[1] From Johns Hopkins University Circulars, vol. ix., No. 81, p. 72.

DESCRIPTION OF APPARATUS FOR PHOTOMICROGRAPHY BY OIL LIGHT.

The apparatus consists of a camera, hung in a vertical position, of a microscope with substage condensers, suitable objectives and projection oculars, and a Laverne tri-wick, oil stereopticon with the projection objective removed.

The Light.—After trying all kinds of lamps, I found that the best illumination could be obtained by using a tri-wick stereopticon with the projection objective removed, the middle wick only being lighted. The large four-inch condensers serve to concentrate the light, while the double lantern body prevents the radiation of heat to the microscope and shuts off all radiating light. Consequently the microscope does not become heated, and if the room is darkened the absence of extraneous light greatly aids in focussing on the camera screen. The oil light itself is quite yellow and so nearly monochromatic that with orthochromatic plates a color screen is seldom, if ever, required. After experimenting by taking photographs with and without a screen, I have found no particular difference in result even when photographing difficult bacteria, and now seldom use one. If a screen is used a solution of bichromate of potash and sulphate of copper in dilute ammonia water placed in a trough between the lantern and microscope gives excellent results and does not materially lengthen the time of exposure. The lantern is placed about twelve inches in front of the microscope and with its central long axis in a plane which extends through the centre of the microscope mirror, the substage condenser, the objective, ocular and centre of camera.

Microscope.—The microscope is used in the upright position. I have used this position rather than the horizontal for several reasons. The microscope is used on the work-table in an upright position, and in working when an object is found which it is desired to photograph, the microscope without changing adjustments has only to be carried to the photomicrographic apparatus, placed in position, correct adjustments of light made, the camera racked into contact and the exposure made. With a conveniently placed dark room the whole operation will occupy but a few minutes. The upright position is necessitated when liquid preparations, as colonies of bacteria floating on liquefied gelatin, are to be photographed, or when the microscope is used for clinical photomicrography, as in photographing urinary deposits in urine, blood corpuscles in Thoma blood counter, etc. In bacteriological work where the bacteria are stained on the cover and after mounting the balsam is not quite dry, the cover is apt to slip if the microscope is used horizontally, but this does not occur with the microscope placed vertically. The horizontal position and long extension of camera is necessary for certain work, particularly where large pictures (*i.e.*, over four inches in diameter) have to be taken, or where it is desired to obtain high amplification by extension of camera rather than by high eyepiecing, or in photographing test diatoms with very high amplifications. For practical work, however, up to amplifications of one thousand diameters, and for photographs for illustration or reproduction, which are seldom required of over three and one-half or four inches in diameter, the upright position is much to be preferred on account of its ease of application and its practical advantages.

Camera.—The upright position of the microscope necessitates a similar position for the camera. To allow easy working, the camera is hung on a rack-work attached to a rigid upright. The upright is placed to the right of the microscope so that it will be out of the way while working.

Both the upper and the lower ends of the camera are movable on the rack-work. The upper end, which carries the screen and plate-holder, is movable, in order that different amplifications within limits can be gotten with the same objective. The lower end is movable that it may be racked up and out of the way and allow the operator to manipulate the microscope before attaching the camera. The bellows has an extension of two feet, measured from the eyepiece of the microscope to the focussing screen. This, with a two-millimeter objective and projection ocular 4, gives an amplification of one thousand diameters. With less extension of bellows and lower objectives amplifications ranging down to ten diameters may be obtained. In focussing, the operator can, by standing, observe the image on the screen with a focussing glass and manipulate the fine adjustment of the microscope with his hand without using a focussing rod, though a suitable focussing rod can be easily fastened to the camera upright if desired.

Setting Up the Apparatus.—The camera being hung on the rack-work the microscope is placed beneath it, a stage micrometer is placed on the stage and a medium-power objective and eyepiece attached to the microscope. Light is reflected from the lantern upon the object by the mirror of the microscope, the observer accurately centres the micrometer, then removing the working eyepiece a projection ocular is inserted, the camera racked down, and with the image of the micrometer projected on the camera screen the microscope is moved in such position that the centre of the micrometer image is exactly in the centre of the screen. This position of the microscope is marked once for all, and whenever afterward the microscope is placed in the same place the centre of the object will be projected on the centre of the screen. To correctly place the lantern, a lower-power objective is used, together with a high-power (Abbe) condenser. The objective is accurately focussed on the lines of the stage micrometer; by adjusting the substage condenser a clear image of the lamp flame is projected on the plane of the object (micrometer) and the lantern is moved to such position that the image will be central. If the camera is attached, the image will appear central on the focussing screen.

This position of the lantern, like that of the microscope, should be fixed.

To Photograph.—In photographing by oil light with all but the lowest powers some form of substage condenser is necessary. This is due to the fact that the source of light must always be focussed on the object in order to give proper definition. In working with the objectives of four millimetres or lower, it will be found advantageous to use objectives of lower power as substage condensers, for it will be found that if placed in the substage for ordinary work they greatly improve the definition of objects. In fact it may be laid down as a general rule that whatever with a given light gives the best definition to the observer's eye will give the sharpest photographic image. Consequently, in high-power work where a condenser is used it will seldom be necessary to change the microscope attachments when a photograph has to be taken; for in bacteriological work the Abbe condenser which gives good definition will, when properly adjusted, give good photographic definition also, statements to the contrary notwithstanding.

To photograph, place the microscope and lantern in position, light the centre wick of the lamp, place a ground glass between the lamp and camera, and focus the objective accurately on the object. The ground glass is used only to reduce the light which might otherwise injure the observer's eye.

The ground glass is then removed, a fine wire screen placed close against the front of the lantern condenser, and by means of the substage condenser an image of the screen is projected accurately on the object. This is very important, for it is necessary that the light should be accurately focussed on the object in order to produce sharp definition. After focussing the light, the screen is removed and an opal glass is put in its place. On looking through the eyepiece a clear sharp image of the object will be seen. If an Abbe condenser is used the iris diaphragm of the condenser should now be carefully opened and closed until such an aperture is obtained that to the observer's eye the object appears to the best advantage. The opal glass is now removed, the camera attached to the microscope, and the projected image focussed on the camera screen, preparatory to exposure.

Plate Used.—Orthochromatic plates only should be used. Of these I use the Cramer rapid, isochromatic plate exclusively. With these when photographing bacteria and using an amplification of one thousand diameters the exposure will vary from one and one-half to three minutes, two minutes being about the average.

It is with these plates that I have found a color screen unnecessary, and since using them I have had no difficulty in photographing bacteria, for they are particularly sensitive to the yellow-colored oil light.

Possibly other makes of orthochromatic plates might be found to work equally well, but the oil light works so very well with the Cramer isochromatic that I have had no desire to try others.

Development.—For development, I have obtained best results with formulas in which hydrochinon either alone or with some other developing agent is used. The following gives excellent results, and I prefer it to other developers as it gives good clear negatives of sufficient contrast and gradation:

No. 1.

Water,	10 ounces.
Sodium sulphite,	1 ounce.
Potassium bromide,	10 grains.
Hydrochinon,	30 grains.
Metol,	40 grains.

No. 2.

Water,	10 ounces.
Sodium carbonate,	300 grains.

Use equal parts of No. 1 and No. 2.

Development should be continued until sufficient density is obtained. Intensification should be rarely required, for with proper exposure and development a good negative can usually be obtained. If intensification is necessary, after fixing and washing the plate, I prefer to use a saturated aqueous solution of bichloride of mercury, followed by washing, the application of dilute ammonia water, and a final washing.

Students who desire to perfect themselves in the art of making photomicrographs are advised to first make themselves familiar with the technique of photography with a landscape or portrait camera, and not to undertake the more difficult task of photographing bacteria until they know how to make a good negative and to judge whether an exposure has been too long or too short, etc.

PLATE I.

PHOTOMICROGRAPHS OF BACTERIA MADE BY GASLIGHT.

FIG. 1.—Streptococcus cadaveris, from a culture in agua coco; stained with fuchsin. × 1,000. (Sternberg.)

FIG. 2.—Streptococcus Havaniensis. × 1,000. From a photomicrograph. (Sternberg.)

FIG. 3.—Bacillus cuniculicida Havaniensis, from peritoneal cavity of inoculated rabbit, showing leucocytes containing bacilli and free bacilli; stained with fuchsin. × 1,000. (Sternberg.)

FIG. 4.—Bacillus cadaveris, smear preparation from yellow-fever liver kept for forty-eight hours in an antiseptic wrapping (Havana, 1889); stained with fuchsin. × 1,000. (Sternberg.)

Note.—All of the above photomicrographs were made with the three-millimetre apochromatic hom. ol. im. objective and projection eye-piece of Zeiss.

PLATE II.

PHOTOGRAPHS OF COLONIES (IN ESMARCH ROLL TUBES) AND OF TEST-TUBE CULTURES.

FIG. 1.—Colonies of Bacillus leporis lethalis, in gelatin roll tube, end of forty-eight hours at room temperature. × 5. (Sternberg.)

FIG. 2.—Colonies of Bacillus coli similis in gelatin roll tube, end of twenty-four hours at 22° C. × 10. (Sternberg.)

FIG. 3.—Stick culture of Bacillus coli similis in nutrient gelatin, end of seven days at 20° C. (Sternberg.)

FIG. 4.—Stick culture of Bacillus intestinus motilis in nutrient gelatin, end of four days at 22° C. (Sternberg.)

FIG. 5.—Stick culture of Bacillus leporis lethalis in nutrient gelatin, end of eight days at 22° C. (Sternberg.)

FIG. 6.—Stick culture of Micrococcus tetragenus versatilis in nutrient gelatin, end of two weeks at 22° C. (Sternberg.)

FIG. 7.—Colonies of Bacillus cuniculicida Havaniensis in gelatin roll tube, end of forty-eight hours at 21° C. × 10. (Sternberg.)

FIG. 8.—Colonies of Bacillus coli communis in gelatin roll tube, end of forty-eight hours at 22° C. × 10. (Sternberg.)

PLATE I.

STERNBERG'S BACTERIOLOGY.

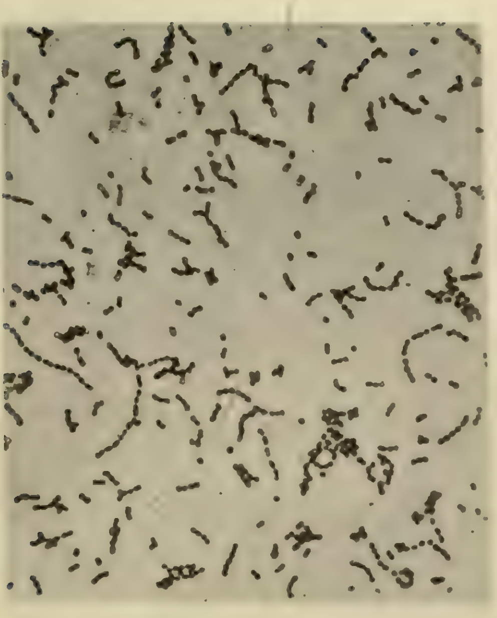

Fig. 1.

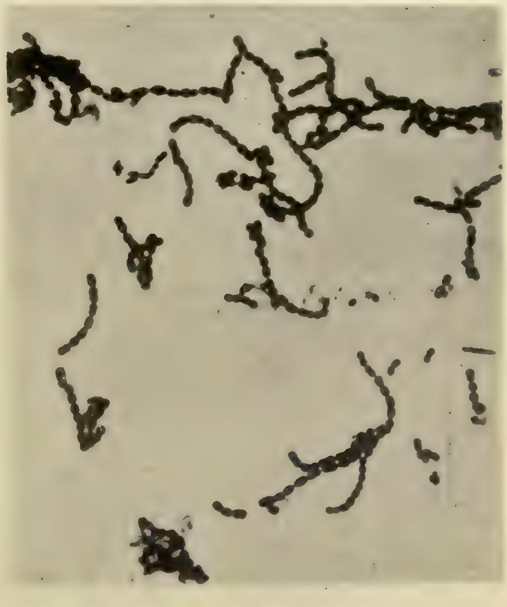

Fig. 2.

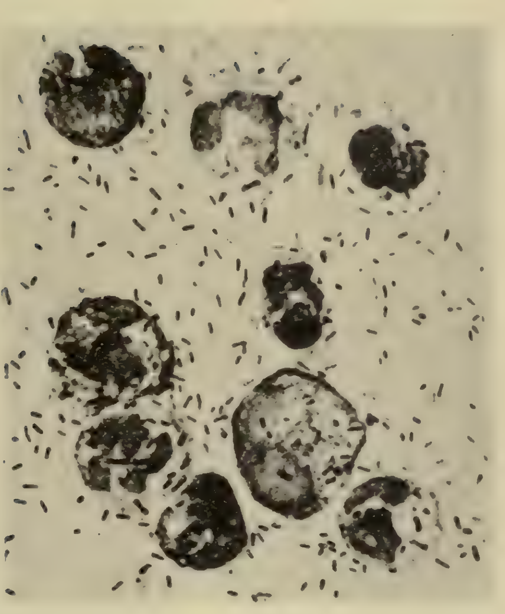

Fig. 3.

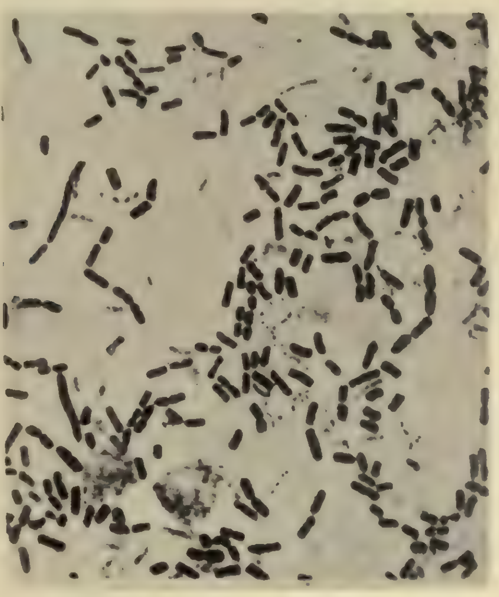

Fig. 4.

PHOTOMICROGRAPHS BY GAS LIGHT.

PLATE II.

STERNBERG'S BACTERIOLOGY.

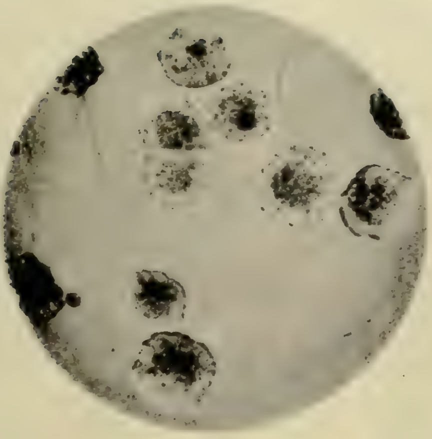

Fig. 1.

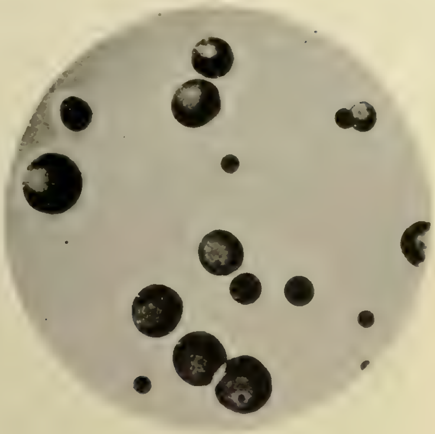

Fig. 2.

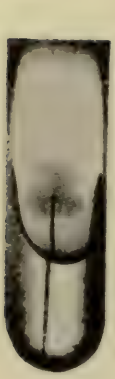

Fig. 3.

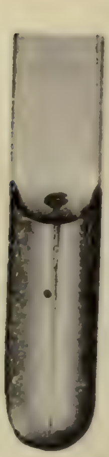

Fig. 4.

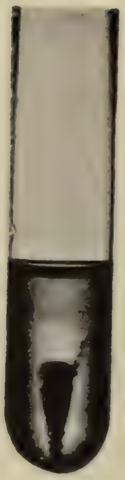

Fig. 5.

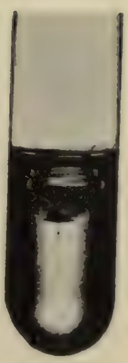

Fig. 6.

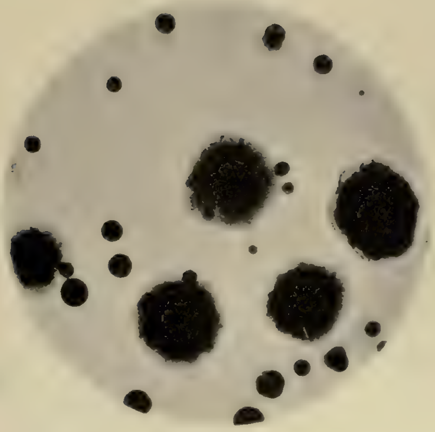

Fig. 7.

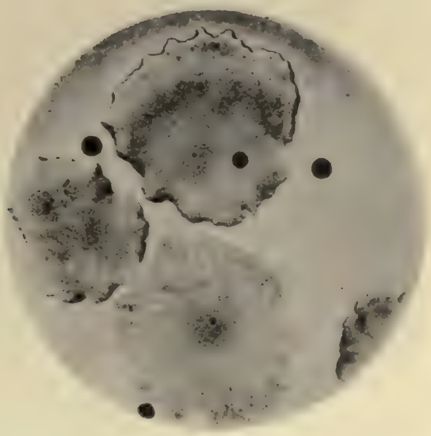

Fig. 8.

COLONIES AND TEST TUBE CULTURES.

PART SECOND.

GENERAL BIOLOGICAL CHARACTERS:

INCLUDING AN ACCOUNT OF THE ACTION OF ANTISEPTICS AND GERMICIDES.

I. Structure, Motions, Reproduction. II. Conditions of Growth. III. Modifications of Biological Characters. IV. Products of Vital Activity. V. Ptomaines and Toxalbumins. VI. Influence of Physical Agents. VII. Antiseptics and Disinfectants—General Account of the Action of. VIII. Action of Gases and of the Haloid Elements upon Bacteria. IX. Action of Acids and Alkalies. X. Action of Various Salts. XI. Action of Coal-tar Products, Essential Oils, etc. XII. Action of Blood Serum and other Organic Liquids. XIII. Practical Directions for Disinfection.

PART SECOND.

I.

STRUCTURE, MOTIONS, REPRODUCTION.

THE bacteria are unicellular vegetable organisms, and consist of a *cell membrane* enclosing transparent and apparently structureless *protoplasm.* The very varied biological characters which distinguish different species make it evident, however, that there are essential differences in the living cell contents, although these differences are not revealed by our optical appliances. And among the bacteria, as in the cells of higher plants and animals, the peculiar biological characters of a species are transmitted to the cellular progeny of each individual cell. These characters are, however, subject to various modifications as a result of differing conditions of environment, as is the case with plants and animals higher in the scale of existence, and in this way more or less permanent varieties are produced. It is probable that among these lowly plants species are evolved more quickly, as a result of the laws of natural selection, in the struggle for existence, than among those of more complex organization. Still, this has not been proved, and, on the other hand, we have ample evidence that widely distributed species exist having very definite morphological and biological characters which enable us to recognize them wherever found.

It has generally been supposed that these simple vegetable cells are destitute of a nucleus, but a recent author (Fränkel) suggests the probability that a nucleus may exist, although it has not been demonstrated. This suggestion is based upon the fact that in staining bacteria very quickly it sometimes happens that a portion of the protoplasm is sharply differentiated by taking the stain more deeply than the remaining portion.

Sjöbring in 1892 made an investigation for the purpose of ascertaining the structure of bacterial cells. Various methods were employed, but the most satisfactory results were obtained by fixing with nitric acid, with or without alcohol, and without previous drying; the preparations were then stained with carbol-methylene-blue or carbol-fuchsin solution; they were decolorized with nitric acid and examined in glycerin or in water. By this procedure the author named was able to demonstrate two kinds of corpuscles. One of these may be seen just inside the cell wall; it stains deeply with the carbol-fuchsin solution. The other lies in a position analogous to that occupied by the nucleus of vegetable cells higher in the scale, and resembles this both in its resting condition and in the process of indirect division.

In his address before the International Medical Congress of Berlin (1890) Koch says:

"We had not succeeded, in spite of the constantly improving methods of staining and in spite of the use of objectives with constantly increasing angles of aperture, in learning more with reference to the interior structure of the bacteria than was shown by the original methods of staining. Only very recently new methods of staining appear to give us further information upon the structure of the bacteria, inasmuch as they serve to differentiate an interior portion of the protoplasm, which should probably be regarded as a nucleus, from an exterior protoplasmic envelope from which is given off the organ of locomotion, the flagellum."

Although usually transparent, the protoplasm sometimes presents a granular appearance. The botanist Van Tieghem claims to have found chlorophyll grains in some water bacteria studied by him, and in the genus Beggiatoa grains of sulphur are found embedded in the protoplasm of certain species.

The granules in bacterial cells which may be demonstrated by special methods of staining are of two kinds: *metachromatic granules* and *polar granules.* The former lie in the protoplasm, and when properly stained may present the appearance of a short chain of cocci lying in the bacterial cell. To demonstrate their presence Ernst recommends the use of Löffler's solution of methylene blue. This is placed upon a cover-glass preparation and heated over a flame until steam begins to rise. After washing in water the cover glass is placed for a minute or two in a watery solution of Bismarck brown. This shows the granules stained blue and the surrounding protoplasm brown. The polar granules are often seen in preparations stained in the usual way with an aniline staining solution. Some observers have regarded these stained granules as spores, but this has not been demonstrated, and cultures containing them show no greater resistance to heat or to chemical agents than that established for the vegetative cells of the particular species in which they are found. It seems probable that the matachromatic granules result from degenerative changes rather than that they are reproductive bodies.

The cell membrane in certain species appears to be very flexible, as may be seen in those which have a sinuous motion. It is not easily recognized under the microscope, but by the use of reagents which cause the protoplasm to contract may be demonstrated—*e.g.*, by iodine solution. Outside of the true cell membrane a gelatinous envelope—so-called capsule—is sometimes seen. This may perhaps be, as claimed by some authors, nothing more than a jelly-like thickening of the outer layers of the cell wall. This jelly-like material causes the cells to adhere to each other, forming zoöglœa masses. In some cases the growth upon the surface of a culture medium is extremely viscid, and may be drawn out into long threads when touched with a platinum needle, owing to the gelatinous intercellular substance by which the cells are surrounded.

There is but little more to be said of the structure of these minute organisms, except to mention the fact that the motile species are provided with slender, whip-like appendages called *flagella.* The micrococci in general are not endowed with the power of executing spontaneous movements, and they are not provided with flagella. But recently two motile species have been described, and in one of these—Micrococcus agilis of Ali-Cohen—the presence of flagella has been demonstrated.

Many of the bacilli and spirilla are actively motile, and the presence of flagella, which has long been suspected, has recently been demonstrated for a considerable number of species by Löffler and others.

It must be remembered that the molecular movement which is common to all minute particles suspended in a fluid is a vibratory motion *in situ,* which does not change the relative position of the moving particles. This so-called Brownian movement has frequently been mistaken for a vital motion, as has also the movement due to currents in the liquid in which non-motile organisms are suspended. The latter is to be distinguished by the fact that the microörganisms are all carried in one direction. This movement due to a current, in connection with the vibratory Brownian movement, is very deceptive, and it is often hard for a beginner in bacteriological study to convince himself that what he sees is not a vital movement. But in true vital movements we have progression in different directions, and the individual microörganisms approach and pass each other, often in a most vigorous and active manner, passing entirely across the field of view or changing direction in an abrupt way. Sometimes the motion is slow and deliberate, the bacillus progressing with a to-and-fro motion, as if propelled by a trailing flagellum ; or it may be serpentine when the moving filament is flexible ; or again it is a darting forward motion which is so rapid that the eye can scarcely follow the moving body. The spirilla have a rotary movement as well as a progressive one, and this is often extremely rapid. Sometimes bacilli spin around with a rotatory motion, as if they were anchored fast to a fixed point, as they may be by the flagellum being attached to the slide or cover glass. Frequently, in a pure culture, the individual bacilli may be seen to come to rest, and, after an interval of repose, to dart forward again in the most active way. Or we may find, on examining the same culture at different times, that upon one occasion there is no evidence of vital movements, and on another all of the bacilli are actively motile. These differences depend upon the age of the culture, temperature conditions, etc.

Reproduction by binary division is common to all of the bacteria, and in many species this is the only mode of reproduction known. When circumstances are favorable for rapid multiplication the individual cells grow in length, and a constriction occurs in the middle transverse to the long diameter. This becomes deeper, and after a time the cell is completely divided into two equal portions, which again divide in the same way. Separation may be complete, or the cells may remain attached to each other, forming chains (streptococci) or articulated filaments (*scheinfäden* of the Germans).

The bacilli and spirilla divide only in a direction transverse to the long diameter of the cells, but among the micrococci division may occur either in one direction, forming chains; or in two directions, forming tetrads; or in three directions, forming "packets" of eight or more elements. The staphylococci, in which the cells do not remain associated, divide indifferently in any direction.

The rapidity of multiplication by binary division varies greatly in different species, and in the same species depends upon conditions relating to the culture medium, age of the culture, temperature, etc. Under favorable conditions bacilli have been observed to divide in twenty minutes, and it is a matter of common laboratory experience that colonies of considerable size and containing millions of bacilli may be developed from a single cell in twenty-four to forty-eight hours. A simple calculation will show what an immense number of cells may be produced in this time as a result of binary division occurring, for example, every hour. The progeny of a single cell would be at the end of twenty-four hours 16,777,220, and at the end of forty-eight hours the number would be 281,500,000,000.

Some of the earlier observers have noted the presence of oval or spherical refractive bodies in cultures containing bacilli; but that these were reproductive elements, although suspected, was not demonstrated until a comparatively recent date. Pasteur was one of the first to point out the fact that certain bacteria have two modes of reproduction—by fission and by the formation of *endogenous spores*; but the first careful study of the last-mentioned method was made by

Koch in his classical study of the anthrax bacillus (1878), and by Cohn, who studied the formation of spores in Bacillus subtilis.

These reproductive bodies serve the same purpose in the preservation of species as the seeds of higher plants. They resist desiccation and may retain their vitality for months or years until circumstances are favorable to their development, when, under the influence of heat and moisture, they reproduce the vegetative form—bacillus or spirillum—with all of its biological and morphological characters. They are composed of condensed protoplasm which retains the vital characters of the soft protoplasm of the mother cell from which it has been separated; and it is evident that whether reproduction occurs by fission or by the formation of endogenous spores, the protoplasm of the cells in a pure culture of any microörganism is simply a separated portion of the protoplasm of the progenitors of these cells.

Some of the bacilli grow out into long filaments before the formation of spores occurs; and these filaments may be associated in bundles or intertwined in irregular masses. At first the protoplasm of the filaments is homogeneous, but after a time it becomes segmented, and later the protoplasm of each segment becomes condensed into a spherical or oval refractive body, which is the spore. For a time these are retained in a linear position by the cell membrane of the filament (Fig. 75, *a*), but this is after a while dissolved or broken up and the spores are set free. In liquid cultures they sink to the bottom as a pulverulent precipitate, and upon the surface of a solid medium they form a layer which is usually of a white or yellowish-white color, and which, when examined under the microscope, in old cultures is found to consist almost entirely of shining spherical or oval bodies which do not stain, by the ordinary methods, with the aniline colors. While many of the bacilli during the stage of spore formation grow out into long filaments, others do not, and one or more spores make their appearance in rods of the ordinary length which characterizes the species. These may be located in the centre of the rod or at one extremity (Fig. 75, *b*). It sometimes occurs that when a single central spore is formed the rod becomes very much enlarged in its central portion, assuming a spindle shape (Fig.

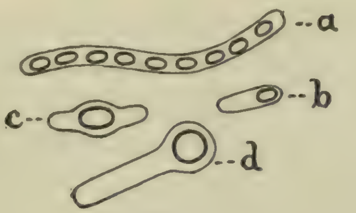

Fig. 75.

75 *c*); or one extremity may be enlarged, producing forms such as are shown in Fig. 75, *d*. Some of the smaller spherical spores measure less than 0.5 μ in diameter, but they are, for the most part, oval bodies having a short diameter of 0.5 to 1 μ and a long diameter of one to two μ, or even more. They are enveloped in a cellular envelope which, according to some observers, consists of two layers—an exosporium and an endosporium.

The mode of spore formation shown in Fig. 75, *c* and *d*, has been adopted by some authors as a generic character. When the spores are located in the central part of the rods, giving rise to a spindle-shaped body, as at *c*, the bacilli are assigned to the genus *Clostridium*; when located at one end, as at *d*, the bacilli are shaped like a drumstick, and this mode of spore formation is used as the distinguishing characteristic of the genus *Plectridium*. Hueppe groups all rod-shaped bacteria which form endospores under the generic name *Bacillus*, with three sub-genera: *Bacillus*, straight rods; *Clostridium*, spindle-shaped rods; *Plectridium*, drumstick-shaped rods.

The germination of spores has been studied by Prazmowski, Brefeld, and others. The process is as follows: By the absorption of water they become swollen and pale, losing their shining, refractive appearance. Later a little protuberance is seen upon one side or at one extremity of the spore, and this rapidly grows out to form a rod which consists of soft-growing protoplasm enveloped in a membrane which is formed of the endosporium or inner layer of the cellular envelope of the spore. The outer envelope, or exosporium, is cast off and may be seen in the vicinity of the newly formed rod (Fig. 76). Sometimes the vegetative cell emerges from one extremity of the oval spore, as shown at *a*, Fig. 76, and in other species the exosporium is ruptured and the bacillus emerges from the side, as seen at *b*.

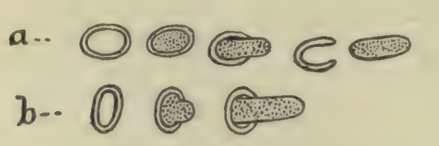

FIG. 76.

The considerable resistance of these endogenous spores to desiccation, to heat, and to various chemical agents is an important fact both from a biological and from a hygienic point of view, and will be fully considered in a subsequent chapter. The fact that certain bacilli and spirilla do not withstand a temperature of 80° to 90° C., which does not destroy the vitality of known spores, leads to the inference that they do not form similar reproductive bodies. But reproductive elements of a different kind are described by some botanists as being produced during the development of these bacteria, and also of the micrococci. These are the so-called *arthrospores*. In the process of binary division certain cells in a chain may be observed to be somewhat larger than others and to refract light more strongly. The same may be true of certain cells in a culture in which the elements are not united in chains. These cells are believed by De Bary and others to have greater resisting power to desiccation than the remaining cells in the culture, and to serve the purpose of reproductive elements.

Hueppe groups all rod-shaped bacteria which do not form endospores under the generic name *Arthrobacterium*. This author believes that, as a rule, bacilli which do not form endospores under certain circumstances produce more resistant cells which "take charge of the perpetuation of the species under the guise of a resting stage or spore." According to Hueppe, true arthrospores are spherical in form.

It has generally been supposed that spore formation is most likely to occur when the pabulum for supporting the growth of the vegetative form is nearly exhausted. But, as pointed out by Fränkel, facts do not support this view, as many species form spores when conditions are most favorable for a continued development. An abundant supply of oxygen favors the formation of spores in aërobic species, and, in some instances at least, the temperature has an important influence upon spore formation. Thus the anthrax bacillus does not form spores at temperatures below 20° C. or above 42° C.

The very interesting fact has been demonstrated by Lehman and by Behring that a species which usually forms spores may be so modified by certain influences that it is no longer capable of spore production, and that such an asporogenous variety may be cultivated for an indefinite time without showing any return to the stage of spore formation. This was effected in Behring's experiments by cultivating the anthrax bacillus in a medium containing some agent detrimental to the vitality of the vegetative cells, but not in sufficient quantity to restrain their development.

The pseudo-branching of the filaments in the genus *Cladothrix* has been referred to in the chapter on *Morphology*. Recent researches show that other bacteria heretofore included in the genus *Bacillus* may also present branching forms. This is especially true of the tubercle bacillus, which when obtained from cases of fowl tuberculosis not infrequently exhibits a sort of branching. Hueppe and Fischel have also demonstrated the presence of branching forms of the bacillus of mammalian tuberculosis, and as a result of his observations Hueppe has "arrived at the definite opinion that the tubercle bacillus is the parasitic growth-form of a pleomorphic mould, and is not a true bacterium at all." Metschnikoff has reported his observations of branching forms of the cholera spirillum, Fränkel of the diphtheria bacillus, and Semmer of the bacillus of glanders, but whether these are examples of pseudo-branching, such as occurs in the genus *Cladothrix*, or a veritable dichotomous growth such as occurs in the mould fungi, has not been definitely determined.

The *chemical composition* of the bacterial cells has been investigated by Nencki, Brieger, and others. Putrefactive bacteria cultivated in a two-per-cent solution of gelatin, and which produced an abundant intercellular substance connecting the cells in zoöglœa masses, were found by Nencki to have the following composition: Water, 84.26 per cent; solids, 5.74 per cent, consisting of albumin 87.46 per cent, fat 6.41, ash 3.04, undetermined remnant 3.09. The albuminous substance, according to Nencki, is not precipitated by alcohol, and differs in its chemical composition from other known substances of this class. He calls it *mykoprotein* and gives the following as its chemical composition: C, 52.32 per cent; H, 7.55 per cent; N, 14.75 per cent. It contains no sulphur and no phosphorus. The spores of the anthrax bacillus, according to Nencki, do not contain mykoprotein, but a peculiar albuminous substance which he calls anthrax-protein. Brieger analyzed a gelatin culture of Friedländer's bacillus, with the following result: Water, 84.2 per cent; solids, 5.8 per cent, containing 1.74 per cent of fats. After removal of the fat the solids gave an ash of 30.13 per cent; this contains calcium phosphate, magnesium phosphate, sodium sulphate, and sodium chloride. The amount of nitrogen in the dried substance after removal of the fat was 9.75.

II.

CONDITIONS OF GROWTH.

BACTERIA only grow in presence of moisture, under certain conditions of temperature, and when supplied with suitable pabulum. As they do not contain chlorophyll, they cannot assimilate carbon dioxide, and light is not favorable to their development.

The aërobic species obtain *oxygen* from the air and cannot grow unless supplied with it. The anaërobic species, on the other hand, will not grow in the presence of oxygen, and must obtain this element, as they do carbon and nitrogen, from the organic material which serves them as food.

As a class the bacteria are supplied with nutriment by the higher plants and animals, the dead tissues of which they appropriate, and which it is their function to decompose, releasing the organic elements as simple compounds which may again be assimilated by the chlorophyll-producing plants.

Water is essential for the development of bacteria, and many species have their normal habitat in the waters of the ocean, of lakes, and of running streams ; others thrive upon damp surfaces or in the interior of moist masses of organic material. Many species grow indifferently either in salt or fresh water, but it is probable that certain species will be found peculiar to the waters of the ocean. Some of the water bacteria multiply in the presence of an exceedingly minute amount of organic pabulum, or even in distilled water. This is shown by the experiments of Bolton and others. The author named tested two species of water bacteria (Micrococcus aquatilis and Bacillus erythrosporus) in the following manner : Ten cubic centimetres of distilled water in a test tube were infected with a small quantity of a culture of one of these microörganisms. A drop from this tube was transferred to the same quantity of distilled water in a second tube, and from this to a third. The number of bacteria in this tube No. 3 was now ascertained by counting, and it was put aside for two or three days, at the end of which time the number was again estimated by counting. In every case there was an enormous increase in the number of bacteria. In order to be sure that the distilled water was pure, it was distilled a second time in a clean glass retort, but the result was the same. Bolton remarks, with reference to these results: "If we seek to explain this remarkable fact we must remember, in the first place, what an extremely small absolute mass is represented by an enormous number of bacteria, and what a minute amount of material is required for the formation of this mass. In ten cubic centimetres of distilled water, in the experiment last referred to, there were about twenty million bacteria (two million per cubic centimetre). If we estimate the diameter of each at one μ, with a specific weight of 1, the absolute weight would be for the entire number one-one-hundredth of a milligramme—that is to say, a quantity which cannot be determined by any of our methods of weighing."

Bolton supposes that the small amount of organic pabulum required fell into the water in the shape of dust, or was attached to the walls of the test tube in spite of all the precautions taken.

Nitrogen is chiefly obtained from albuminoid substances, but Pasteur has shown that it may also be obtained from ammonia. This is shown by cultivating bacteria in a medium containing an ammonia salt, as in the following:

PASTEUR'S SOLUTION.

Distilled water,	100
Cane sugar,	10
Tartrate of ammonia,	1
Ashes of one gramme of yeast,	0.075

COHN'S SOLUTION.

Distilled water,	100
Tartrate of ammonia,	1
Ashes of yeast,	1

Many bacteria multiply abundantly in these solutions.

Carbon is obtained from the various organic substances containing it; among others, from starch, sugars, glycerin, organic acids and their salts, etc.

Temperature.—There are certain limits of temperature within which development may take place, but these differ greatly with different species. As a rule, growth is arrested when the temperature falls below 10° C. (50° F.), but some species multiply at a still lower temperature. Thus Bolton observed a very decided increase in certain water bacteria kept in an ice chest at 6° C., and other observers have witnessed development at the freezing temperature.

Most saprophytic bacteria grow within rather wide temperature limits, but the rapidity of development is greatest at a certain favorable temperature, which is usually between 25° and 30° C. The parasitic species have a more restricted range, which approaches the normal temperature of the animals in which they habitually develop. At 40° C. (104° F.) growth, as a rule, ceases, but there are some notable exceptions to this rule.

Miquel some years ago found a bacillus in the water of the Seine which grew at a temperature of 69° to 70° C.; Van Tieghem reports having observed species in thermal waters capable of growth at a still higher temperature (74° C.); and Globig has more recently obtained from garden earth several species which multiplied at 65° C. Some of the species found by the last-named observer were even found to require a temperature of about 60° for their development; and yet this temperature is quickly fatal to a large number of the best known species.

Low temperatures, while arresting the growth of bacteria, do not destroy their vitality. This has been demonstrated by numerous experiments, in which they have been exposed for hours in a refrigerating mixture at $-18°$ C. Frisch has even subjected them to a temperature of $-87°$ C. by the evaporation of liquid carbon dioxide, and found that they still grew when placed in favorable conditions.

Parasitism.—The strict parasites grow only in the bodies of living animals, or in artificial media kept at a suitable temperature. As examples we may mention the bacillus of tuberculosis, the bacillus of leprosy, the micrococcus of gonorrhœa, the spirillum of relapsing fever. There is also a large class of *facultative parasites* which, when introduced into the body of a susceptible animal, multiply in it, and may continue to live as parasites so long as they are transferred from one animal to another, but which are also able to live as saprophytes independently of a living host. To this class belong the pus cocci, the bacillus of typhoid fever, the spirillum of cholera, and many others.

It seems extremely probable that the strict parasites were at one time capable of living a saprophytic existence, and that their restriction to a parasitic mode of life has been effected in course of time in accordance with the laws of natural selection. This view is supported by the fact that the tubercle bacillus, which has been regarded as a strict parasite, which can only be cultivated artificially under very special conditions, has been shown to be capable of modification in this regard to such an extent that when cultivated for a time in a favorable medium—bouillon with five per cent of glycerin—it will even grow in ordinary bouillon made from the flesh of a calf or a fowl (Roux).

Reaction of Medium.—Some bacteria grow readily in a medium having an acid reaction, while the slightest trace of acidity prevents the development of others. As a rule, the pathogenic species require a neutral or slightly alkaline culture medium.

While many species grow in various media and under various conditions of temperature, etc., others are greatly restricted in this regard; thus Bumm only succeeded in cultivating the gonococcus upon *human blood serum,* and even upon this was not able to carry it through a series of successive cultures. It is very probable that certain species can only grow in association with others which elaborate products necessary for their development.

Substances favorable for the growth of a particular species may restrain its development if present in too large an amount. Thus the phosphorescent bacilli multiply abundantly in a nutrient solution containing 2.5 per cent of sodium chloride; but this amount would restrain the development of some other species, and a considerable increase in the quantity of salt prevents the growth of all microörganisms. In the same way the addition of two per cent of glucose to culture solutions is favorable for the development of certain species, and especially for the anaërobic bacteria; but a concentrated solution of the same substance prevents the growth of all bacteria.

The influence of one species upon the growth of another has been studied by various bacteriologists, and especially by Sirotinin and by Freudenreich. When several species are associated in the same culture one may take the precedence and the others may develop later; or two or more species may develop at the same time; or the growth of one species may prevent the development of another, either (*a*) by exhausting the pabulum necessary for its growth or (*b*) by producing substances which inhibit the development of another species or destroy its vitality.

Freudenreich found, as a result of his numerous experiments, that the following species cause a change in bouillon which renders it unfit for the growth of other species: Bacillus pyocyaneus, Bacillus cyanogenus, Bacterium phosphorescens, Bacillus prodigiosus, Spirillum choleræ Asiaticæ. The following species do not cause such a change in bouillon as to render it unfit for the growth of other species: Bacillus typhi abdominalis, Bacillus anthracis, Bacillus septicæmiæ hæmorrhagicæ, Spirillum tyrogenum. The following have a decided antagonism: Bacillus pyogenes fœtidus prevents the growth of Spirillum choleræ Asiaticæ; Micrococcus roseus prevents the growth of Micrococcus tetragenus. The cholera spirillum will not grow in sterilized cultures of Bacillus pyocyaneus, or in bouillon which has served for a previous culture of the same microörganism (Kitasato). Other bacteria which fail to grow in bouillon which has already served for the cultivation of the same species are Bacillus typhi abdominalis, Bacillus cyanogenus, Bacillus prodigiosus, Micrococcus roseus, etc. (Freudenreich).

III.

MODIFICATIONS OF BIOLOGICAL CHARACTERS.

We have already referred to the production of an asporogenous variety of the anthrax bacillus. This was effected by Behring by cultivation in media containing small amounts of hydrochloric acid, caustic soda, methyl violet, malachite green, and various other agents. This is only one of many instances of a change in biological characters due to changed conditions of environment. We have abundant experimental evidence that growth may occur under adverse conditions when the species is gradually habituated to these conditions. Thus the temperature limitations may be passed by successive cultivations at temperatures approaching these limits, and bacteria may grow in the presence of agents which in a given proportion have a complete restraining influence upon their development. For example, in the experiments of Kossiakoff, published in the *Annales* of the Pasteur Institute (vol. i.), it was found that the several species tested all became habituated to the presence of antiseptic agents in proportions which at first completely restrained their growth.

This modification of biological characters is well shown in the case of the chromogenic bacteria, some of which only form pigment under exceptionally favorable conditions of growth. It has been shown by several observers that non-chromogenic varieties of some of the best known chromogenic species may be produced by special methods of cultivation. Thus Wasserzug obtained a non-chromogenic variety of the bacillus of green pus (Bacillus pyocyaneus) by the action of time added to that of antiseptics. He says: "These two actions combined have permitted me to obtain cultures which remained without color in a durable way, and in which, consequently, the chromogenic function was abolished by heredity." In the case of a chromogenic bacillus obtained by the writer in Havana (my Bacillus Havaniensis), a non-chromogenic variety was obtained from a culture on nutrient agar which had been kept in a hermetically sealed glass tube for about a year. The variety preserved the morphological characters of the original stock, but, although carried through successive cultures for a considerable period, did not regain its power to produce the brilliant carmine color which is the most striking character of the species. Katz, in cultivating the phosphorescent bacilli isolated by him from sea water at New South Wales, found that, after being propagated for some time in artificial media, their power to give off a phosphorescent light was diminished or temporarily lost. He also found that two species which when first cultivated did not liquefy gelatin, subsequently, after a year, caused liquefaction of the usual gelatin medium.

Modification shown in Cultures.—When bacteria have been subjected to the action of heat or chemical agents, without having their vitality completely destroyed, they often show diminished vigor of growth. Cultures which would ordinarily show an abundant development within twenty-four hours may not commence to grow for several days. For this reason, in disinfection experiments, it is necessary to test the question of destruction of vitality by leaving the cultures for a week or more under favorable conditions as to temperature. In plate cultures or Esmarch roll tubes a few colonies may develop in this tardy way, showing that there was a difference in the vital resisting power of the individual cells, some having survived while the majority were killed. This is well illustrated by Abbott's experiments upon the germicidal action of mercuric chloride as tested upon Staphylococcus pyogenes aureus. Irregularities in the results in experiments in which the conditions were identical having been noticed, Abbott inferred that this was due to a difference in the resisting power of individual cocci (arthrospores ?). By making cultures from colonies which developed from these more resistant cocci, and again exposing the micrococci in these cultures to mercuric chloride in the proportion of 1:1,000 for a longer time and making new cultures from the surviving cocci, and so on, Abbott obtained cultures in which a majority of the cells survived exposure to a solution of the strength mentioned for ten to twenty minutes, whereas in his original culture most of the cocci were killed by this solution in five minutes.

These changes in vital resisting power enable us to comprehend other modifications which can only be detected by chemical or biological reactions. Thus the reducing power for various substances may be modified by changes in the conditions of environment. And among the pathogenic bacteria changes of a more or less permanent nature may be induced, which are shown by a modified degree of virulence when injected into susceptible animals.

Attenuation of Virulence may be effected by several methods, all of which depend upon subjecting the cultures to prejudicial influences of one kind or another.

Pasteur first announced, in 1880, that the microbe of fowl cholera could be modified by special treatment in such a manner that it no longer produced a fatal form of the disease. He found that the virulence was greatest when cultures were made from fowls which had died from a chronic form of the disease, and that this virulence was not lost by successive cultivations in chicken bouillon, repeated at short intervals. But when an interval of more than two months was allowed to elapse without renewing the cultures, the virulence was diminished and fewer deaths occurred in fowls inoculated with such cultures. This diminution of virulence became more marked in proportion to the length of time during which a culture solution containing the microbe remained exposed to the action of the atmosphere, and at last all virulence was lost as a result of the death of the pathogenic microörganism. When the virus was preserved in hermetically sealed tubes it did not undergo this modification, but retained its full virulence for many months. According to Pasteur, the various degrees of modification of virulence resulting from prolonged exposure to the air may be preserved in successive cultures made at short intervals. Subsequent experiments with cultures of the anthrax bacillus gave similar results and enabled him to produce an "attenuated virus" for his protective inoculations.

In the case of the anthrax bacillus it was found that the spores retain their full virulence for years, and that the production of an attenuated virus required the exclusion of these reproductive elements. Cultivations were consequently made at a temperature of 42° to 43° C., at which point this bacillus is incapable of producing spores. Cultivation at this temperature for eight days gave an attenuated virus suitable for use in protective inoculations.

Attenuation by Heat.—Toussaint has shown that a similar modification of virulence may be produced by exposure for a short time to a temperature a little below that which destroys the vitality of the pathogenic organism. This is best accomplished, according to Chauveau, in the case of the bacillus of anthrax, by exposure for eighteen minutes to a temperature of 50° C. Exposure to this temperature for twenty minutes is said to completely destroy the vitality of the bacillus.

Attenuation by Antiseptic Agents.—The writer, in 1880, obtained evidence that attenuation of virulence may result from exposure to the action of antiseptic agents. In a series of experiments made to determine the comparative value of disinfectants, the blood of a rabbit recently dead from a form of septicæmia induced by the subcutaneous injection of my own saliva, and due to the presence of a micrococcus (Micrococcus pneumoniæ crouposæ), was subjected to the action of various chemical agents, and subsequently injected into a rabbit to test the destruction of virulence. In the published report of these experiments the following statement is made :

"The most important source of error, however, and one which must be kept in view in future experiments, is the fact that a protective influence has been shown to result from the injection of virus the virulence of which has been modified, without being entirely destroyed, by the agent used as a disinfectant."

"Sodium hyposulphite and alcohol were the chemical reagents which produced the result noted in these experiments; but it seems probable that a variety of antiseptic substances will be found to be equally effective when used in proper proportion. Subsequent experiments have shown that neither of these agents is capable of destroying the vitality of the septic micrococcus in the proportion used (one per cent of sodium hyposulphite or one part of ninety-five-per-cent alcohol to three parts of virus), and that both have a restraining influence upon the development of this organism in culture fluids."[1]

Cultivation in the Blood of an Immune Animal.—It has been shown by the experiments of Ogata and Jasuhara that when the anthrax bacillus is cultivated in the blood of an immune animal, such as the dog or the white rat, its pathogenic power is modified so that it no longer kills susceptible animals and may be used as a vaccine.

Pasteur had previously shown (1882) that the virus of rouget can be attenuated by passing it through rabbits.

Recovery of Virulence.—Pasteur has shown that when the virulence of a pathogenic organism has been modified it may be restored by successive inoculations into susceptible animals. Thus in the case of the anthrax bacillus a culture which would not kill an adult guinea-pig may be inoculated into a very young animal of the same species with a fatal result; and by inoculating the blood of this animal into another, and so on, the original virulence may be restored, so that a culture is obtained which will kill a sheep. In the same way the attenuated virus of fowl cholera may be restored to full vigor by inoculating a small bird—sparrow or canary—to which it is fatal. After several successive inoculations the virus resumes its original activity.

In general, pathogenic virulence is increased by successive inoculations into susceptible animals, and diminished by cultivation in artificial media under unfavorable conditions. Thus various pathogenic bacteria which have been cultivated in laboratories for a length of time are likely to disappoint the student if he makes inoculation experiments for the purpose of demonstrating their specific action as described in text books.

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[1] Quoted from "Bacteria," pages 207, 208, written in 1883.

IV.

PRODUCTS OF VITAL ACTIVITY.

All living cells, animal or vegetable, while in active growth, appropriate certain elements for their nutrition from the pabulum with which they are supplied, and at the same time excrete certain products which, in some cases at least, it is their special function to produce. In the higher plants and animals specialized cells excrete substances which are injurious to the economy of the individual, and secrete substances which are required to maintain its existence. As an example in animals we may mention the excretion of urea by the epithelium of the kidneys, the retention of which is fatal to the individual, and the gastric secretion which is essential for its continued existence. Among the higher plants we have an immense variety of substances formed in the cell laboratories, some of which are evidently useful for the preservation of the species, while others are perhaps to be considered simply as excretory products. The odorous volatile products given off by flowers are supposed to be useful to the plant in attracting insects by which cross-fertilization is effected. The various poisonous substances stored up in leaves and bark may serve to protect the plant from enemies, etc.

The minute plants with which we are especially concerned also produce a great variety of substances, some of which may be useful to the species in the struggle for existence. Thus the deadly ptomaines produced by some of the pathogenic bacteria serve to paralyze the vital resisting power of living animals and enable the parasitic invader to thrive at the expense of its host. In the present section we shall consider in a general way these various products of bacterial growth.

Pigment Production.—A considerable number of species are distinguished by the formation of pigment of various colors and shades. We have all of the shades of the spectrum from violet to red. The color, as a rule, is only produced in the presence of oxygen, and when the pigment-producing microörganisms are massed upon the surface of a solid culture medium the pigment production is often limited to the superficial portion of the mass. In some cases a soluble pigment is formed which is absorbed by the transparent culture medium, coloring especially the upper portion, in stab cultures in nutrient gelatin or agar. This is the case with Bacillus pyocyaneus, which produces a blue pigment which has been isolated and carefully studied by Gessard and others. The pigment, which is called pyocyanin, is soluble in chloroform and crystallizes from a pure solution in long blue needles. Acids change the blue color to red, reducing substances to yellow. It resembles the ptomaines in its chemical reactions, being precipitated by platinum chloride and phosphomolybdic acid.

In some media the color produced by the Bacillus pyocyaneus (bacillus of green pus) is a fluorescent green. The recent studies of Gessard show that this is a different pigment. According to this author, cultures in a two-per-cent solution of peptone give a beautiful blue tint, the production of which is hastened by adding to the liquid five per cent of glycerin. In nutrient gelatin and agar cultures a fluorescent green color is developed, which, according to Gessard, is due to the presence of albumin. Peptone and gelatin are said to produce pyocyanin without the fluorescent-green pigment, and cultures in bouillon to give both this and pyocyanin. In milk the fluorescent-green color is first seen, but subsequently, when the casein has been peptonized by a diastase produced in the culture, pyocyanin is also formed. Several other microörganisms are known which produce a fluorescent-green color, due probably to the same pigment as is produced by the bacillus of green pus in albuminous media.

Babes claims to have obtained two pigments from cultures of the Bacillus pyocyaneus in addition to pyocyanin: one, soluble in alcohol, has by transmitted light a chlorophyll-green color, by reflected light it is blue; the other, insoluble in alcohol and chloroform, by transmitted light is of a dark orange-red, by reflected light a greenish-blue.

In Gessard's latest publication (1891) he shows that the production of pyocyanin or of the fluorescent-green pigment does not depend alone upon the culture medium, but that there are different varieties of the Bacillus pyocyaneus. He has succeeded in producing four distinct varieties—one which produces both pyocyanin and fluorescence, one which produces pyocyanin alone, one which produces the fluorescent-green pigment alone, and one which produces no pigment. The last-mentioned non-chromogenic variety was produced by subjecting the second variety to the action of heat. A temperature of 57° maintained for five minutes destroyed the power to produce pigment without destroying the vitality of the bacillus, which was propagated through successive cultures without regaining this power.

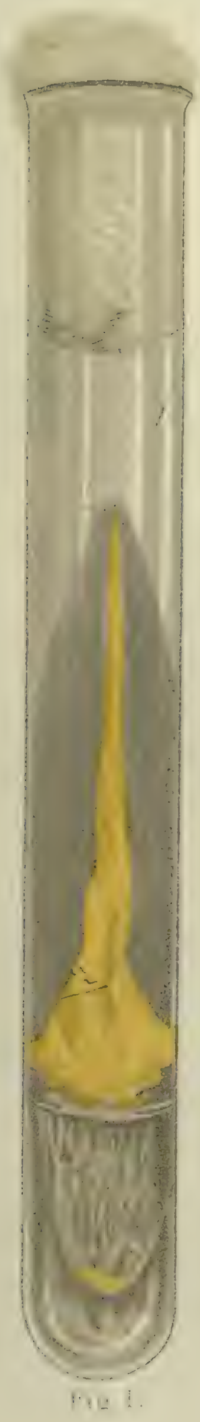

Fig 1.

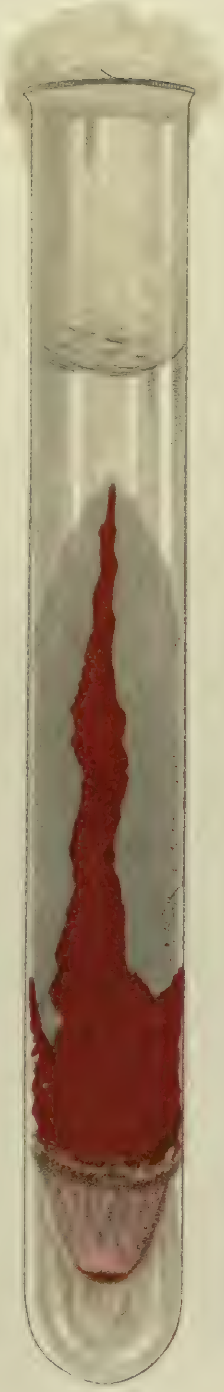

Fig. 2.

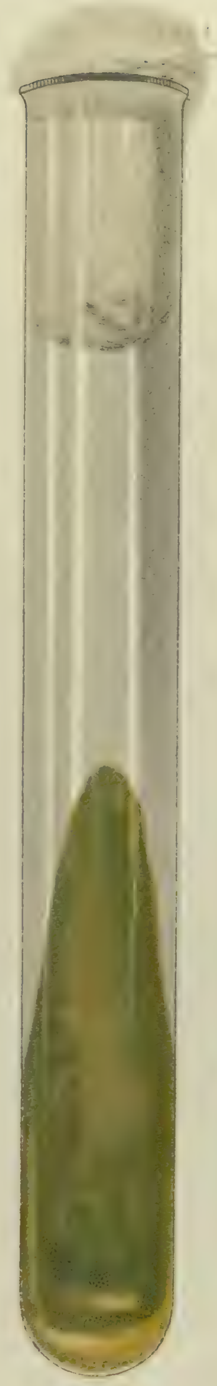

Fig 3.

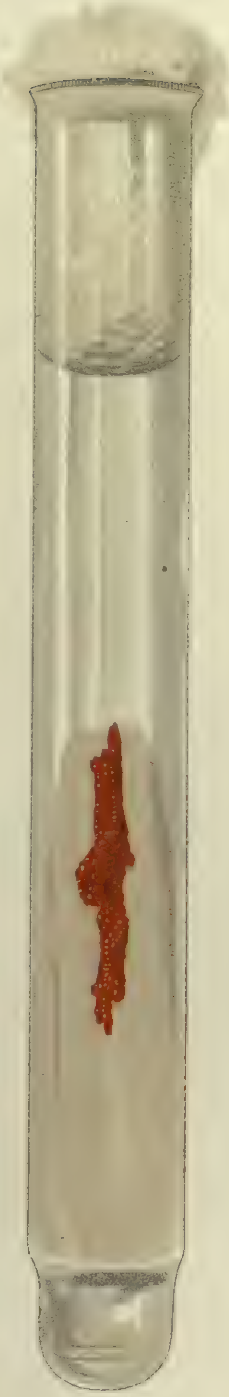

Fig 4.

Fig 1. Sarcina lutea; agar culture
Fig 2. Bacillus prodigiosus agar culture
Fig 3. Bacillus pyocyanus; agar culture
Fig 4. Bacillus Havaniensis potato culture

The well-known Bacillus prodigiosus (also described as a micrococcus) produces a red pigment which is insoluble in water but soluble in alcohol. By the addition of an acid the color becomes carmine and then violet, which is changed to yellow by an alkali. The color is said by Schottelius to be diffused in the young cells, and after the death of the cells to be present in their vicinity in the form of granules. The same author has shown that by subjecting the bacillus to special conditions a variety may be obtained which no longer produces pigment.

The conditions which govern the formation of pigment in the chromogenic bacteria are determined with comparative facility because the results of changed conditions are apparent to the eye; in the case of products which are not colored the difficulties attending the study of these conditions are much greater, but the results are in many instances more important. The following are among the best known pigment-producing (chromogenic) bacteria:

Staphylococcus pyogenes aureus, Staphylococcus pyogenes citreus, Sarcina aurantiaca, Sarcina lutea, Bacillus cyanogenus, Bacillus janthinus, Bacillus fluorescens liquefaciens, Bacillus indicus, Bacillus pyocyaneus, Bacillus prodigiosus, Spirillum rubrum.

Liquefaction of Gelatin.—Many species of bacteria, when planted in a medium containing gelatin, cause a liquefaction of the gelatin in the immediate vicinity of the growing microörganisms, while many others multiply abundantly in the same medium without liquefying the gelatin. This character, as first shown by Koch, is an important one in the differential diagnosis of species which resemble each other in form and in other respects. It has no relation to pathogenic power, as some liquefying organisms are harmless saprophytes and some deadly disease germs, while, on the other hand, non-liquefying bacteria may be very pathogenic or quite innocent.

Liquefaction is produced by a soluble peptonizing ferment formed during the growth of the cells. This is shown by the fact that if a liquefying organism is cultivated in bouillon and the living cells removed by filtration or killed by heat, the power of liquefying gelatin remains in the culture fluid. This was first observed by Bitter (1886) and independently by the writer in 1887. In experiments made to determine the thermal death-point of various bacteria the writer found that when cultures of liquefying species were subjected to a temperature which killed the microörganisms, a few drops of the culture added to nutrient gelatin which had been liquefied by heat prevented it from subsequently forming a solid jelly when cold.

In a study of the ferments produced by bacteria which cause liquefaction of gelatin—"tryptic enzymes"—made by Fermi, in the laboratory of the Hygienic Institute of Munich (1891), the following results were obtained:

The enzymes were not obtained pure, and their isolation from other proteids present in the cultures was found to be attended with great difficulties, but their ferment action was studied and was found to be influenced by various conditions.

All were destroyed by a temperature of 70° C., but the enzymes produced by various liquefying bacteria differed considerably as to the temperature which they were able to withstand. Some were destroyed by a temperature of 50° to 55° C.—Bacillus megatherium, Bacillus ramosus, Staphylococcus pyogenes aureus; some by a temperature of 55° to 60° C.—Bacillus subtilis, Bacillus pyocyaneus, Bacillus fluorescens liquefaciens, Sarcina aurantiaca; some by 65° to 70° C.—Bacillus anthracis, Spirillum choleræ Asiaticæ, Spirillum of Finkler and Prior, Spirillum tyrogenum.

These enzymes, like the previously known pepsin, trypsin, and invertin, do not dialyze.

Only a few of these bacteria enzymes acted upon fibrin, and no action was observed upon casein or upon egg albumen.

Their liquefying action upon gelatin was prevented by the action of sulphuric acid, and to a less degree by nitric acid, but was not interfered with by acetic acid.

The liquefying bacteria, as a rule, only produce enzymes when cultivated in a medium containing albumen.

These enzymes are not produced by a solution of the protoplasm of dead bacterial cells, but are a product of the vital activity of living cells.

Among the numerous liquefying bacteria known to bacteriologists we may mention the following species as deserving the student's special attention: Staphylococcus pyogenes aureus, Staphylococcus pyogenes albus, Sarcina lutea, Sarcina aurantiaca, Bacillus anthracis, Bacillus pyocyaneus, Bacillus subtilis, Bacillus indicus, Bacillus prodigiosus, Spirillum choleræ Asiaticæ, Spirillum of Finkler and Prior, Proteus vulgaris.

Fermentation.—The fermentation produced by various species of bacteria in culture solutions containing saccharose, glucose, or lactose constitutes a valuable character for the differentiation of species. While some bacteria give rise to fermentation in solutions containing either of the carbohydrates above mentioned, others break up lactose, but have no effect upon glucose or saccharose, and others again are without any ferment action. The gases evolved are chiefly carbon dioxide and hydrogen. Ferment action may be tested by adding one to two per cent of glucose to a solid culture medium—preferably agar-agar. This is liquefied by heat in the test tube containing it and a small quantity (one öse) of the microörganism to be tested is introduced. The culture medium is then quickly solidified by placing the test tube in iced water. It is then placed in the incubator, and when colonies form bubbles of gas will be seen in their vicinity, if the bacterium under observation is able to cause fermentation of glucose. For accurate observations as to the quantity and nature of the gases produced the fermentation tube should be used, as recommended by Theobald Smith (see Fig. 38).

Production of Acids.—Numerous bacteria give an acid reaction to the media in which they are cultivated, and the acids produced are various—lactic, acetic, butyric, propionic, succinic, etc.

The power to produce an acid is well shown by adding to neutral or alkaline culture media a solution of litmus. The change in color due to the formation of an acid may be followed by the eye, and comparative tests may be made to aid in the differentiation of similar bacteria.

A considerable number of bacteria are able to produce *lactic acid* from milk sugar and other carbohydrates. One of these is considered the special lactic-acid ferment—Bacillus acidi lactici—and is the usual cause of the acid fermentation of milk. Pure cultures of this bacillus introduced into sterilized milk or solutions of milk sugar, cane sugar, dextrin, or mannite, give rise to the lactic-acid fermentation, in which carbonic acid is also set free. The process requires free access of oxygen, and progresses most favorably at a temperature of 35° to 40° C., ceasing at about 45°. In milk, coagulation of the casein occurs within fifteen to twenty-four hours after adding a small quantity of a pure culture of the lactic-acid bacillus. This is not due, however, to the acid fermentation, but to a ferment resembling that of rennet, which is produced by many different bacteria, some of which do not produce an acid reaction of the milk. Among the bacteria which produce lactic acid from milk sugar we may mention the staphylococci of pus, Bacillus lactis aërogenes, and Bacillus coli communis.

The formula showing the transformation of sugar into lactic acid is usually stated as follows : $C_6H_{12}O_6 = 2(HC_3H_5O_3)$.

Acetic acid is also produced from dilute solutions of alcohol by the action of a special bacterial ferment, which accumulates upon the surface of the fluid as a mycoderma, consisting almost entirely of the Bacillus aceticus (Mycoderma aceti). Free access of oxygen is required, and a temperature of about 33° C. is most favorable to the process. According to Duclaux, the "Mycoderma aceti" oxidizes the alcohol, in solutions containing it, so long as any is present, and when it is exhausted it oxidizes the acetic acid previously formed by oxidation of the alcohol, producing from it carbon dioxide and water.

The formation of acetic acid from alcohol is shown by the following formula : Ethyl alcohol $CH_3.CH_2.OH + O_2 = CH_3.COOH + H_2O$.

Butyric acid is produced by a considerable number of bacteria, one of which, named Bacillus butyricus, has received the special attention of Prazmowski. This is strictly anaërobic. In solutions of starch, dextrin, sugar, or salts of lactic acid, when oxygen is excluded it produces butyric acid in considerable quantity, and at the same time carbon dioxide and hydrogen gas are set free. Duclaux gives the following formula of a solution containing lactate of lime in which the action of the butyric-acid ferment may be well studied :

Water, ...	8 to 10 litres.
Lactate of lime (pure), ...	225 grammes.
Phosphate of ammonia, ...	0.75 "
Phosphate of potash,.	0.4 "
Sulphate of magnesa, ...	0.4 "
Sulphate of ammonia, ...	0.2 "

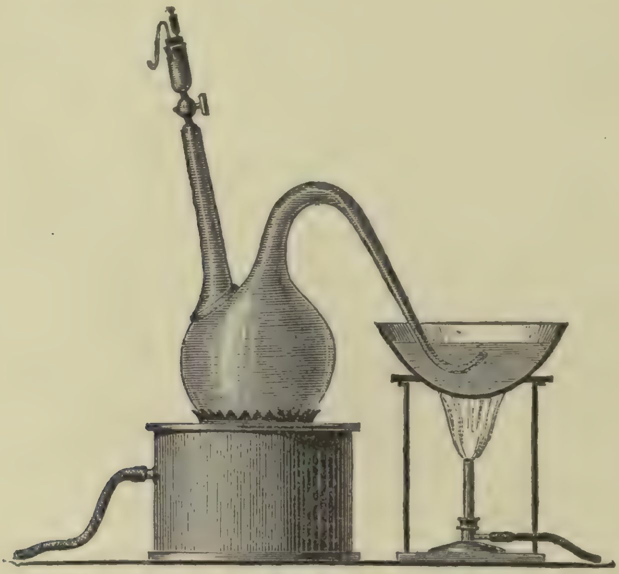

FIG. 77.

This is introduced into a flask with two necks, such as is shown in Fig. 77. Having filled the flask with the culture liquid, the bent neck is dipped into a porcelain dish containing the same. Heat is then applied both to flask and dish, and the liquid in each is kept in ebullition for half an hour. By this means the air is completely driven out of the flask. This is now allowed to cool, while the fluid in the shallow dish is kept hot, so that the liquid mounting from it into the flask shall be free from air. When the flask is full it is transferred to an incubating oven heated to 25° to 30° C., and the bent tube is immersed in a dish containing mercury. The little funnel attached to the upright tube is then filled with carbon dioxide and a culture of the butyric-acid bacillus is introduced into the funnel. By turning the stopcock in the upright tube a little of the culture is admitted to the flask without admitting any air. Fermentation commences very soon, as is seen by the bubbles of gas given off. The liquid loses its transparency and the lactic acid is gradually consumed, butyrate of lime taking the place of the lactate.

Aërobic bacilli capable of producing butyric acid in culture solutions containing grape sugar or milk sugar have also been described by Liborius and by Hueppe.

Fitz has shown that in culture solutions containing glycerin the Bacillus pyocyaneus produces butyric acid in addition to ethyl alcohol and succinic acid. Bacillus Fitzianus also produces some butyric acid in solutions containing glycerin, although the principal product of the fermentation caused by this microörganism is, according to Fitz, ethyl alcohol, twenty-nine grammes of which may be obtained from one hundred grammes of glycerin.

Botkin (1892) has described a "Bacillus butyricus" (No. 466) which he has not been able to identify positively with the butyric-acid ferment described by Prazmowski. It is a widely distributed anaërobic bacillus, which he was able to obtain from milk or water containing it by placing it in the steam sterilizer for half an hour. The spores resisted this temperature and subsequently grew in anaërobic cultures, in a suitable medium, while all other bacteria and spores present were destroyed.

The writer has described a bacillus which causes active acid fermentation in culture solutions containing glycerin. The acid formed is volatile and is probably propionic acid—see Bacillus acidiformans.

The Caucasian milk ferment—Bacillus Kaukasicus—produces a variety of products in the fermented milk which is a favorite drink among the Caucasians. The principal ones are ethyl alcohol, lactic acid, and carbon dioxide, but in addition to these small quantities of succinic, butyric, and acetic acids are formed. The inhabitants of the Caucasian mountains prepare this fermented drink in a very simple manner from the milk of cows or goats, to which they add the dried ferment collected from a receptacle in which the fermentation had previously taken place. Flügge gives the following directions for the preparation of this drink :

> "Two methods may be employed. In the first the dry brown *kefir-körner* of commerce are allowed to lie in water for five to six hours until they swell; they are then carefully washed and placed in fresh milk, which should be changed once or twice a day until the *körner* become pure white in color and when placed in fresh milk quickly mount to the surface—in twenty to thirty minutes. One litre of milk is then poured into a flask and a full tablespoonful of the prepared *körner* added to it. It is allowed to stand

open for five to eight hours; the flask is then closed and kept at 18° C. It should be shaken every two hours. At the end of twenty-four hours the milk is poured through a fine sieve into another flask, which must not be more than four-fifths full. This is corked and allowed to stand, being shaken from time to time. At the end of twenty-four hours a drink is obtained which contains but little CO_2 or alcohol. Usually it is not drunk until the second day, when, upon standing, two layers are formed, the lower milky, translucent, and the upper containing fine flakes of casein. When shaken it has a cream-like consistence. On the third day it again becomes thin and very acid.

"The second method is used when one has a good *kefir* of two or three days to start with. Three or four parts of fresh cow's milk are added to one part of this and poured into flasks which are allowed to stand for forty-eight hours with occasional shaking. When the drink is ready for use a portion (one-fifth to one-third) is left in the flask as ferment for a fresh quantity of milk. The temperature should be maintained at about 18° C.; but at the commencement a higher temperature is desirable. The *körner* should be carefully cleaned from time to time and broken up to the size of peas. The cleaned *körner* may be dried upon blotting paper in the sun or in the vicinity of a stove: when dried in the air they retain their power to germinate for a long time."

Fermentation of urea. The alkaline fermentation of urine is effected by various microörganisms, but chiefly by the Micrococcus ureæ, the ferment action of which has been carefully studied by Pasteur, Duclaux, and others. The change which occurs under the action of the living ferment was determined by the chemist Dumas as long ago as 1830, but it remained for Pasteur to show that this change depends upon the presence and vital activity of a living microörganism.

The transformation of urea into carbonate of ammonia is shown by the following formula: $COH_4N_2 + 2H_2O = CO_2 + 2NH_3 + H_2O = (NH_4)_2CO_3$.

According to Van Tieghem, Micrococcus ureæ continues to grow in a liquid containing as much as thirteen per cent of carbonate of ammonia. It may be cultivated in an artificial solution of urea, with the addition of some phosphates, as well as in urine.

The Bacillus ureæ of Miquel has also the power of producing the alkaline fermentation of urine, but it does not thrive in so strong a solution of carbonate of ammonia.

A different micrococcus—Micrococcus ureæ liquefaciens—has also been studied in Flügge's laboratory which possesses the same power. According to Musculus, a soluble ferment may be isolated from urine which has undergone alkaline fermentation, which changes urea into carbonate of ammonia. He obtained it from urine containing considerable mucus, in a case of catarrh of the bladder. But Leube has shown that cultures of Micrococcus ureæ from which the micrococcus was removed by filtration through clay do not induce alkaline fermentation. The soluble ferment obtained by Musculus must therefore be from some other source.

Miquel has given special attention to the study of bacteria which produce alkaline fermentation in urine, and in addition to the species above mentioned has described the following : Urobacillus Pasteuri, Urobacillus Duclauxi, Urobacillus Freudenreichi, Urobacillus Maddoxi, Urobacillus Schutzenbergi.

Viscous fermentation. A special fermentation which occurs sometimes in wines, and in the juices of bulbous roots containing glucose, and in milk, is produced by various bacteria. One of these is a micrococcus which has been described by Conn under the name of Micrococcus lactis viscosus. The fermented juices become very viscous, owing to the formation of a gum-like product resembling dextrin; at the same time mannite and CO_2 are produced. The gum-like substance, called viscose by Béchamp, is soluble in cold water and is precipitated by alcohol. Guillebeau (1892) has described a micrococcus and a bacillus which produce viscous fermentation in milk—Micrococcus Freudenreichi and Bacillus Hessi. A micrococcus producing viscous fermentation in milk has also been described by Schmidt-Mühlheim, and a bacillus by Löffler. Bacillus mesentericus vulgatus also produces a similar change in milk.

Marsh gas, CH_4, is produced by the fermentation of cellulose, through the action of microörganisms the exact characters of which have not yet been determined. According to Tappeiner, there are two different fermentations of cellulose. The first occurs in a neutral one-per-cent flesh extract solution to which cotton or paper pulp has been added. The gases given off are CO_2 and CH_4 and small quantities of H_2S. The second fermentation occurs when an alkaline solution of flesh extract containing cellulose in suspension is used. The gases formed are CO_2 and H. In both cases small quantities of aldehyde, isobutyric acid, and acetic acid are produced.

Hydrosulphuric acid, H_2S. This gas is produced during the growth of certain bacteria. The conditions governing its development have been studied by Holschewnikoff, who experimented with two species, one isolated by himself and one by Lindenborn, named respectively Bacterium sulfureum and Proteus sulfureus. The first-mentioned bacterium, when inoculated into eggs, produced within three or four days an abundant quantity of H_2S ; the other did not. Upon raw albumin both species produced but little, and on the yolk of egg a considerable amount of this gas. Upon cooked egg the action was the reverse. In peptone-bouillon the evolution of H_2S was abundant ; in the absence of peptone, very slight.

Putrefactive fermentation. The putrefactive decomposition of albuminous material of animal and vegetable origin is effected by a great variety of microörganisms and gives rise to the formation of a great variety of products, some of which are volatile and are characterized by their offensive odors. According to Flügge, the first change which occurs consists in the transformation of the albumins into peptone, and this may be effected by a large number of different bacteria. Among the products of putrefactive fermentation known to chemists are the following substances : Carbon dioxide, hydrogen, nitrogen, hydrosulphuric acid (H_2S), phosphoretted hydrogen (PH_3), methane, formic acid, acetic acid, butyric acid, valerianic acid, palmitic acid, crotonic acid, glycolic acid, oxalic acid, succinic acid, propionic acid, lactic acid, amidostearic acid, leucin, ammonia, ammonium carbonate, ammonium sulphide, trimethylamine, propylamine, indol, skatol, tyrosin, neuridin, cadaverin, putrescin, cholin, neurin, peptotoxin, and various other volatile acids, ptomaines, etc.

The special products of putrefaction vary according to the nature of the material, the conditions in which it is placed, and the microörganisms present. One or the other of the bacteria concerned will take the precedence when circumstances favor its growth. Thus the aërobic bacteria cannot grow unless the putrefying material is freely exposed to atmospheric oxygen ; the anaërobic species require its exclusion. Some saprophytic bacteria grow at a comparatively low temperature, others take the precedence when the temperature is high ; some, no doubt, thrive only in presence of products evolved by other species, and are consequently associated with and dependent upon these species ; some are restrained in their growth sooner than others by the products evolved as a result of their own vital activity or that of associated organisms ; some grow in the presence of acids and give rise to an acid fermentation which wholly prevents the development of other species.

At the outset putrefaction is often attended with the presence of several species of micrococci and certain large bacilli, which are displaced later by short motile bacteria belonging to a group which includes several bacilli formerly described under the common name of Bacterium termo.

The malodorous volatile products of putrefaction are to a considerable extent produced by anaërobic species. For this reason these odors are more pronounced when masses of albuminous material undergo putrefaction in situations where the oxygen of the air has not free access or where it is displaced by carbon dioxide. The body of a dead animal, although freely exposed to the air, furnishes in its interior a suitable nidus for these anaërobic gas-forming species, and they may give rise to products of one kind, while aërobic species upon the surface of the mass induce different forms of putrefactive fermentation. In the bodies of living animals these anaërobic microörganisms are constantly present in the intestine, and after death they quickly invade the body and multiply at its expense under favorable conditions as to temperature. The surface decomposition due to aërobic bacteria occurs later and is not attended with the same putrefactive odors, the products evolved being of a simpler chemical composition—CO_2, HN_3. No doubt these aërobic bacteria, by consuming the oxygen and forming an atmosphere of carbon dioxide, help to make the conditions favorable for the continued development of the anaërobics in the interior of the organic mass ; at the same time they find a suitable pabulum in some of the more complex products of decomposition occurring in the absence of oxygen. The gases produced in the interior of a putrefying mass are mainly CH_4, H_2S, and H.

Many of the bacteria of putrefaction are facultative anaërobics—that is to say, they are able to multiply either in the presence of oxygen or in its absence. The products evolved by these differ, no doubt, according to whether they are or are not supplied with atmospheric oxygen.

The anaërobic bacteria concerned in putrefaction have as yet received comparatively little attention. Among the aërobics and facultative anaërobics the following are best known: Micrococcus fœtidus, Bacillus saprogenes I., II., and III., Bacillus coprogenes fœtidus, Bacillus putrificus coli, Proteus vulgaris, Proteus Zenkeri, Proteus mirabilis, Bacillus pyogenes fœtidus, Bacillus fluorescens liquefaciens, Bacillus pyocyaneus, Bacillus coli communis, Bacillus janthinus.

Soluble Ferments.—Several species of bacteria produce soluble ferments capable of changing starch into maltose, dextrin, etc. Hueppe has shown that the lactic-acid bacillus produces a diastase, and Miller obtained from the human intestine a species which dissolves starch. Marcano, by filtering cultures of species capable of this ferment action through porcelain, was able to show that the effect is due to a soluble ferment, which must have been produced by the vital activity of the living microörganisms. Wortmann also obtained a diastase from culture liquids which was precipitated by alcohol and again dissolved in water ; in slightly acid solutions it promptly converted starch into glucose. This is said to be produced in culture liquids only when these do not contain albumin. In the presence of albumin a peptonizing ferment was formed ; in its absence, a diastase by which starch was dissolved to serve as pabulum for the bacteria present. These experiments were not made with pure cultures, and more exact researches in this direction are desirable.

A peptonizing ferment for gelatin is produced by a considerable number of bacteria, as stated under the heading "Liquefaction of Gelatin." The jellified albumin in cultures in blood serum is also liquefied by a peptonizing ferment produced by certain species of bacteria.

Some authors also speak of a soluble ferment capable of inverting cane sugar or milk sugar. According to Hueppe, such a ferment is produced by the Bacillus acidi lactici. A soluble ferment for cellulose is supposed by Flügge to be produced by several species—among others by Bacillus butyricus and by Vibrio rugula.

Several bacilli produce a soluble ferment capable of coagulating the casein of milk.

Reduction of Nitrates, and Nitrification.—The researches of Gayon, Dupettit, and others show that certain bacteria are able to reduce nitrates with liberation of ammonia and free nitrogen. This is effected in the absence of oxygen by anaërobic bacteria, and, among others, by Bacillus butyricus. Certain aërobic bacteria also accomplish the same result. Thus Heræus obtained two species from water which reduced nitrates in a very decided manner. On the other hand, a number of species are known to oxidize ammonia, producing nitric acid. Schlösing and Münz, as a result of numerous experiments, arrived at the conclusion that in the soil nitrification is effected by a single species. But it is doubtful whether they worked with pure cultures, and more recent researches show that several, and probably many, different bacteria possess this power. According to Heræus, the following species, tested by him, oxidize ammonia: Bacillus prodigiosus, the cheese spirillum of Deneke, the Finkler-Prior spirillum, the typhoid bacillus, the anthrax bacillus, the staphylococci of pus. The oxidation does not always go to the point of forming nitrates, but nitrites may be formed in the soil (Duclaux). Warrington states that certain bacteria which formed nitrates in a suitable culture medium produced only nitrites when, after an interval of four or five months, some of the culture was transferred to a solution containing muriate of ammonia. The same author states that the process of nitrification occurs only in the dark.

The researches of Winogradsky, of the Franklands, and of Jordan show that the failure of earlier investigators to obtain the nitrifying bacteria from the soil in pure cultures was due to the fact that these bacteria do not grow in the usual culture media. By the use of certain saline solutions the authors named have succeeded in isolating nitrifying bacteria in pure cultures, or nearly so. It is still uncertain whether these investigators have obtained the same bacteria, but the microörganisms described by them, and obtained from widely distant sources, are similar in their morphological and biological characters, and at least belong to the same group. In a communication made in 1891 Winogradsky arrives at the conclusion that the ferments which cause the oxidation of ammonia and production of nitrites are not capable of producing nitrates, but that other microörganisms are concerned in the oxidation of nitrites. In sterilized soil to which a pure culture of his nitromonas was added nitrites only were produced, and the presence of various microörganisms common in the soil did not result in the formation of nitrates so long as the specific ferment was absent to which this second oxidation is ascribed (nitrifying bacillus of Winogradsky).

Phosphorescence.—Several different bacteria have been studied which, in pure cultures, give rise to phosphorescence in the medium in which they are cultivated. In gelatin cultures the light is sufficient in some instances to enable one to tell the time by a watch in a perfectly dark room, and such cultures have even been photographed by their own light.

The phosphorescence is influenced by changes in the culture medium and by conditions of temperature, but we have no exact knowledge of the mode of its production. The Bacillus phosphorescens from sea water in the vicinity of the West Indies gives the most striking results, especially when planted upon the surface of cooked fish and placed in an incubating oven at 30° C. Two other species have been studied by Fischer—one obtained from the water of the harbor at Kiel, and the other a widely distributed species called by Fischer Bacterium phosphorescens. Katz (1891) has described several species obtained by him from sea water and from phosphorescent fish in the markets at Sydney, New South Wales—Bacillus smaragdino-phosphorescens, Bacillus argenteo-phosphorescens, Bacillus cyaneo-phosphorescens, Bacillus argenteo-phosphorescens liquefaciens.

V.

PTOMAINES AND TOXALBUMINS.

VARIOUS basic substances containing nitrogen, and in chemical constitution resembling the vegetable alkaloids, have been isolated by chemists from putrefying material and from cultures of the bacteria concerned in putrefaction, and also from certain pathogenic species. Some of these *ptomaines* are non-toxic, and others are very poisonous in minute doses (*toxines*). The toxic substances sometimes developed in milk, cheese, sausage, etc., are also of this nature, and are doubtless produced by the action of microörganisms. The pathogenic power of the bacteria which cause various infectious diseases in man and the lower animals has also been shown to result from the production of toxic ptomaines or of toxalbumins. Selmi first gave the name ptomaines to cadaveric alkaloids isolated by him, and Panum subsequently called attention to the fact that poisonous basic substances of this class are contained in putrefying material. Extended researches with reference to the ptomaines have since been made by numerous chemists, the most important being those of Bergmann, Schmiedeberg, Zuelzer and Sonnenschein, Hager, Otto, Selmi, Brieger, Gautier and Étard, and Vaughan.

For a full account of the history and chemical composition of the ptomaines the reader is referred to the valuable work of Vaughan and Novy ("Ptomaines and Leucomaines," Philadelphia, 1891). In the present volume we shall give a brief account only of some of the most important.

NON-TOXIC PTOMAINES.

Neuridin, $C_5H_{14}N_2$.—This is one of the most common of the alkaloids of putrefaction and was isolated by Brieger in 1884. It is obtained most abundantly from tissues containing gelatin. Very soluble in water, but insoluble in ether and absolute alcohol. Has a disagreeable odor.

Cadaverin, $C_5H_{14}N_2$.—Isomeric with neuridin; has a very disagreeable odor; forms a thick, transparent, syrupy liquid; is volatile, and can be distilled with steam without undergoing decomposition. When exposed to the air the base absorbs carbon dioxide and forms a crystalline mass. Is produced in cultures of the cholera spirillum and of the spirillum of Finkler and Prior which have been kept for a month or more at 37° C.

Putrescin, $C_4H_{12}N_2$.—A base resembling cadaverin and commonly associated with it. Obtained by Brieger from various sources, most abundantly from substances containing gelatin and in the more advanced stages of putrefaction. It is obtained in the form of a hydrate, which is a transparent liquid having a boiling point of about 135°. With acids it forms crystalline salts.

Saprin, $C_5A_{16}N_2$.—Resembles cadaverin and is commonly associated with it in putrefying material. Isolated by Brieger.

Methylamine, $CH_3.NH_2$.—Obtained by Brieger from putrefying fish and from old cultures of the cholera spirillum.

Dimethylamine, $(CH_3)_2.NH$.—Obtained by Brieger from putrefying gelatin and by Bocklisch from decomposing fish.

Trimethylamine, $(CH_3)_3N$.—Obtained from various sources, and by Brieger from cultures of the cholera spirillum and of the streptococcus of pus.

TOXIC PTOMAINES.

Neurin, $C_5H_{13}NO$. — First obtained by Liebreich in 1865 as a decomposition product of protagon from the brain. Obtained by Brieger from putrefying muscular tissue. When crystallized from an aqueous solution it forms five- or six-sided plates ; from an alcoholic solution it crystallizes in the form of needles (Liebreich). This base is toxic in small doses. In frogs the injection of a few milligrammes produces paralysis of the extremities. Respiration is first arrested and the heart stops in diastole. Atropine appears to be a physiological antidote to the toxic effects of neurin. In rabbits it produces profuse salivation. The pupil is contracted by the direct application of a concentrated solution.

Cholin, $C_5H_{15}NO_2$.—First obtained from hog's bile by Strecker in 1862. Has been obtained by Brieger from various sources, including cultures of the cholera spirillum. It is also found widely distributed in the vegetable kingdom. May be prepared from the yolk of eggs by the method of Diakonow. Cholin is obtained in the form of a syrupy, alkaline liquid which combines with acids to form deliquescent salts. At first this base was not supposed to have toxic properties, but more recent researches have shown that in comparatively large doses it produces symptoms resembling those caused by minute doses of neurin.

Muscarin, $C_5H_{15}NO_3$.—This toxic principle of poisonous mushrooms has also been obtained by Brieger from putrefying fish. It may be produced artificially by the oxidation of cholin. In small doses it kills rabbits and frogs. In the rabbit it produces lacrymation and salivation, the pupil is contracted, and the animal dies in convulsions. Frogs are completely paralyzed by the action of muscarin and die with arrest of the heart's action in diastole.

Peptotoxin.—The exact composition of this ptomaine has not been determined. Brieger obtained it during the early putrefaction of proteid substances and also from the artificial digestion of fibrin. It is very poisonous for frogs, which become paralyzed and die within fifteen or twenty minutes after the subcutaneous injection of a few drops of a dilute solution. Rabbits also are killed by doses of half a gramme to a gramme, the symptoms being paralysis of the posterior extremities and stupor. Peptotoxin is soluble in water, but insoluble in ether or chloroform. It is not destroyed by boiling.

Tyrotoxicon.—First obtained by Vaughan in poisonous cheese, and subsequently by the same chemist and others in poisonous milk and ice cream. Chemically tyrotoxicon is very unstable. It is decomposed when heated with water to 90° C. It is insoluble in ether. From sixteen kilogrammes of poisonous cheese Vaughan obtained 0.5 gramme of the poison. The symptoms produced in man by eating cheese or milk containing tyrotoxicon are vertigo, nausea, vomiting, and severe rigors, with pain in the epigastrium, cramps in the legs, griping pain in the bowels attended with purging, numbness and a pricking sensation in the limbs, and great prostration.

Methyl-guanidin, $C_2H_7N_3$.—Obtained by Brieger from putrefying horseflesh which had been kept at a low temperature for several months. This base was previously known to chemists, having been obtained by the oxidation of creatin. By Bocklisch it has been obtained from impure cultures of the Finkler-Prior spirillum which had been kept for about a month. It is obtained as a colorless mass having an alkaline reaction, and which is quite deliquescent. Brieger gives the following account of the toxic action as tested on guinea-pigs in a dose of 0.2 gramme: The respiration increases in rapidity, the pupils dilate to the extreme limit, the animal has copious discharges of urine and fæces, the extremities become paralyzed, and at the end of about twenty minutes death occurs in convulsions.

Mytilotoxin.—Obtained by Brieger from poisonous mussels. The toxic action resembles that of curare.

Typhotoxin, $C_7H_{17}NO_2$.—Obtained by Brieger from bouillon cultures of the typhoid bacillus which had been kept for a week or more at a temperature of about 37.5° C. In mice and guinea-pigs this base produces salivation, rapid respiration, dilatation of the pupils, diarrhœa, and death in from twenty-four to forty-eight hours. It is believed by Brieger that the specific action of the typhoid bacillus is due to the production of this ptomaine.

A base which is isomeric with typhotoxin has been obtained by

Brieger from putrefying horseflesh which was kept at a low temperature for several months. Unlike it, however, the free base has an acid reaction, while typhotoxin is strongly alkaline. It differs also in its physiological action, being more toxic and producing convulsions; the heart is arrested in diastole. Typhotoxin, on the other hand, does not induce convulsions and the heart is arrested in systole.

Tetanin, $C_{13}H_{30}N_2O_4$.—Obtained by Brieger from impure cultures of the tetanus bacillus cultivated in bouillon in an atmosphere of hydrogen. (The tetanus bacillus is a strict anaërobic.) Obtained subsequently by the same chemist from the amputated arm of a patient with tetanus. This base has been obtained, by crystallization from hot alcohol, in clear yellow plates which are not very soluble in water. The hydrochloride is a deliquescent salt which dissolves readily in alcohol. When injected into guinea-pigs or mice in rather large doses, tetanin first causes the animal to fall into a lethargic condition, followed by increased rapidity of respiration and tetanic convulsions. In guinea-pigs opisthotonos is induced, together with the characteristic tetanic convulsions as seen in animals suffering from tetanus. Three other toxic bases have been obtained by Brieger from cultures of the tetanus bacillus, which cause similar symptoms. One—*tetanotoxin*—is given by Brieger the formula $C_5H_{11}N$. A second base, the composition of which has not been determined, is called *spasmotoxin*.

Cholera Ptomaines.—Brieger has obtained from pure cultures of the cholera spirillum several of the toxic ptomaines heretofore referred to—cadaverin, putrescin, cholin, methyl-guanidin. In addition to these he found two toxic substances which appear to be peculiar products of this microörganism. One induces cramps and muscular tremors in small animals, the other diarrhœa and symptoms of collapse.

Toxalbumins.—Researches by Brieger and Fränkel (1890) show that very toxic substances of a different nature are present in cultures of some of the pathogenic bacteria; these have been designated by the authors named "toxalbumins."

Roux and Yersin had previously shown that filtered cultures of the diphtheria bacillus contain a toxic substance which produces paralysis and death in guinea-pigs and rabbits. This substance has now been obtained in a pure state and its toxic action tested by the authors first named. It is destroyed by a temperature of 60° C., but remains in an active condition in cultures which have been sterilized by several hours' exposure to a temperature of 50°, or in those which have been passed through a clay filter. It is not volatile, and differs essentially from the ptomaines and also from the soluble ferments. It was obtained as a snow-white, amorphous mass which was extremely toxic in its action upon small animals. When injected into guinea-pigs in the proportion of two and one-half milligrammes to one kilogramme of body weight, it caused death after a considerable interval of time (from a few days to several weeks), during which the animal became emaciated and spreading abscesses and necrosis of the tissues occurred at the point of injection. This toxalbumin was obtained in a pure state by repeated precipitation from an aqueous solution by means of alcohol. It is produced most abundantly in cultures containing albumin, and old cultures are more toxic than recent ones. Chemical analysis gave the following result: C 45.35, H 7.13, N 16.33, S 1.39, O 29.80. The authors remark, however, that the chemical characters have not yet been fully determined.

The same chemists have obtained toxic substances of a similar nature from cultures of the bacillus of typhoid fever, of the tetanus bacillus, of the Staphylococcus aureus, and of the cholera spirillum. Hankin had previously obtained a toxic "albumose" from cultures of the anthrax bacillus by precipitation with alcohol, drying, solution in water, and filtration through porcelain; and Christmas had obtained an albuminous substance from cultures of Staphylococcus aureus which produced pus formation when injected beneath the skin of rabbits or into the anterior chamber of the eye.

According to Brieger and Fränkel, these toxalbumins are divided into two principal groups, one of which is characterized by solubility in water, as in that produced by the diphtheria bacillus; and one in which the albumin is insoluble or but slightly soluble, as is the case with those obtained from cultures of the typhoid bacillus, the cholera spirillum, and the Staphylococcus aureus.

The toxalbumin from cholera cultures, obtained as pure as possible and suspended in water, when injected under the skin of a guinea-pig, caused its death in two or three days. It was not, however, toxic for rabbits, even when injected in considerable quantity.

On the contrary, the toxalbumin of the typhoid bacillus, which is dissolved with difficulty in water, was more poisonous for rabbits than for guinea-pigs. When injected subcutaneously into rabbits death usually occurred in eight to ten days. No notable pathological changes were observed at the autopsy.

The toxalbumin of Staphylococcus aureus killed rabbits and guinea-pigs within a few days, and in some cases at the end of twenty-four hours. The post-mortem appearances were necrosis or purulent breaking down of the tissues at the point of injection, with swelling and redness of the surrounding tissues and general inflammatory appearances. The toxalbumin of anthrax cultures resembles that of the diphtheria bacillus in being soluble in water. It was obtained by Brieger from the organs of animals recently dead from anthrax. In a dry condition it has a grayish-white color and gives the reactions of albumins.

The toxalbumin of the tetanus bacillus is also soluble in water. It is best obtained in bouillon cultures containing glucose.

G. and F. Klemperer (1891) have announced their success in obtaining a toxalbumin from cultures of Micrococcus pneumoniæ crouposæ ('diplococcus pneumoniæ'); this they propose to call pneumotoxin.

Some recent authors prefer the name *toxins* for the poisonous products of bacterial growth designated by Brieger and others as "toxalbumins." This avoids any definite statement as to their chemical composition, which appears to be still in doubt. The poisonous precipitates obtained from cultures of the tetanus or the diphtheria bacillus give the reactions of an albumin or albumose (Martin), but it is possible that the toxic substance is simply associated with bodies of this class, and that they have not yet been isolated in a pure state. These toxins in some cases are intimately associated with the bacterial cell—intracellular toxins—and their toxic effects are exhibited when small quantities of dead bacteria are introduced into a susceptible animal. The extracellular toxins are better known, and may be obtained from filtered culture solutions by precipitation with strong alcohol. In this case they are associated with the proteids which may have been present in the culture. The fact that a considerable interval elapses—twenty-four hours to several days—after the injection of these toxins into a rabbit or a guinea-pig before death occurs, has given rise to the inference that these substances are of the nature of enzymes or ferments. This view is also supported by the very minute quantity required to produce a fatal result. According to Vaillard a dose of 0.00025 gramme of the tetanus toxin is sufficient to kill a guinea-pig.

Indol Production.—Numerous species of bacteria, as a result of their vital activity, give rise to the production of indol. This may be detected by cultivation in "Dunham's solution" of peptone (dried peptone, 1 part; sodium chloride, 0.5 per cent; distilled water, 100 parts). Upon adding a drop of yellow nitric acid to ten cubic centimetres of a culture in this medium the presence of indol will be revealed by the development of a rosy red color. The presence of nitrous acid in the *yellow* nitric acid is essential for the reaction, which, however, may be obtained with pure nitric or sulphuric acid if a small quantity of potassium nitrate is added to the culture—one cubic centimetre of a 0.2-per-cent solution.

"*Koch's Tuberculin.*"—This is a glycerin extract of the toxic substances present in cultures of the tubercle bacillus. Crude tuberculin is obtained from liquid cultures made in veal broth to which one per cent of peptone and four to five per cent of glycerin have been added. This culture liquid is placed in flasks and inoculated upon the surface with small masses from a pure culture of the tubercle bacillus. A tolerably thick and dry white layer is developed, which after a time covers the entire surface. At the end of six to eight weeks development ceases and the culture liquid is evaporated over a water bath to one-tenth its volume ; this, after being filtered, constitutes the crude tuberculin. By precipitation with sixty-per-cent alcohol Koch has obtained from this a white precipitate which has the active properties of the glycerin extract. This is soluble in water and in glycerin, and has the chemical reactions of an albuminous body.

Zuelzer has (1891) reported his success in isolating a toxic substance from tubercle cultures. The contents of tubes containing pure cultures of the bacillus are first treated with hot water acidulated with hydrochloric acid. This solution is filtered, evaporated, and then several times precipitated with platinum chloride. The double salt formed is decomposed by hydrosulphuric acid, after which the liquid is filtered and evaporated to dryness. A white, crystalline salt is thus obtained which is soluble in hot water. This salt was toxic for rabbits and guinea-pigs in doses of from one to three centigrammes. Death usually occurred in from two to four days. In guinea-pigs one centigramme injected subcutaneously caused, within a few minutes, a greatly increased frequency of respiration, an elevation of temperature, and protrusion of the eyeballs.

Malleïn.—Kalwing, Preusse, and Pearson have obtained from cultures of the glanders bacillus a "lymph" which somewhat resembles the crude tuberculin of Koch. This was obtained by Preusse by treating old potato cultures of the glanders bacillus with glycerin and water. The extract was filtered several times and then sterilized in a steam sterilizer. This lymph injected into horses infected with glanders gives rise to a very decided elevation of temperature, while in horses free from this disease no such result follows.

VI.

INFLUENCE OF PHYSICAL AGENTS.

Heat.—We have already seen (Section II., Part Second) that the temperature favorable for the growth of most bacteria is between 20° and 40° C.; that some species are able to multiply at the freezing temperature, and others at as high a temperature as 60° to 70° C.; that, as a rule, the parasitic species require a temperature of 35° to 40°; and that low temperatures do not kill bacteria.

Frisch (1877) exposed various cultures to a temperature of −87° C., which he obtained by the evaporation of liquid CO_2, and found that micrococci and bacilli, after exposure to such a temperature, multiplied abundantly when again placed in favorable conditions. Prudden has also made extended experiments upon the influence of freezing. He found that while certain species resisted the freezing temperature for a long time, others failed to grow. Thus Bacillus prodigiosus did not grow after being frozen for fifty-one days; Proteus vulgaris was killed in the same time, and a slender, liquefying bacillus obtained from Croton aqueduct water was killed in seven days. Staphylococcus pyogenes aureus withstood freezing for sixty-six days, a fluorescent bacillus from Hudson River ice for seventy-seven days, and the bacillus of typhoid fever for one hundred and three days. Cultures made at intervals showed, however, a diminution in the number of bacteria. A similar diminution would perhaps have occurred in old cultures in which the pabulum for growth was exhausted, independently of freezing; for bacteria, like higher plants, die in time—which varies for different species—as a result of degenerative changes in the living protoplasm of the cells, and continued vitality in a culture depends upon continued reproduction.

Repeated freezing and thawing was found by Prudden to be more fatal to the typhoid bacillus than continuous freezing. Cultures were sterilized by being thawed out at intervals of three days and again refrozen, after repeating the operation five times.

Cadéac and Malet kept portions of a tuberculous lung in a frozen condition for four months, and found that at the end of this time tuberculosis was still produced in guinea-pigs by injecting a small quantity of this material.

In considering the influence of high temperatures we must take account of the very great difference in the resisting power of the vegetative cells and the reproductive elements known as spores, also of the fact as to whether dry or moist heat is used and the time of exposure.

Dry Heat.—When microörganisms in a desiccated condition are exposed to the action of heated dry air, the temperature required for their destruction is much above that required when they are in a moist condition or when they are exposed to the action of hot water or steam. This was thoroughly demonstrated by the experiments of Koch and Wolffhügel (1881). A large number of pathogenic and non-pathogenic species were tested, with the following general result: A temperature of 78° to 123° C. maintained for an hour and a half (over 100° for an hour) failed to kill various non-pathogenic bacteria, but was fatal to the bacillus of mouse septicæmia and that of rabbit septicæmia. To insure the destruction of all the species tested, in the absence of spores, a temperature of 120° to 128° C., maintained for an hour and a half, was required.

The spores of Bacillus anthracis and of Bacillus subtilis resisted this temperature and required to insure their destruction a temperature of 140° C. maintained for three hours. This temperature was found to injure most objects requiring disinfection, such as clothing and bedding. But the lower temperature which destroys microörganisms in the absence of spores (120° C. = 248° F.) can be used for disinfecting articles soiled with the discharges of patients with cholera, typhoid fever, or diphtheria, as the specific germs of these diseases do not form spores. It is probable also that it may be safely used to disinfect the clothing of small-pox patients, for we have experimental evidence that a lower temperature destroys the virulence of vaccine virus (90°–95° C.—Baxter).

In practical disinfection by means of dry heat it will be necessary to remember that it has but little penetrating power. In the experiments of Koch and Wolffhügel it was found that registering thermometers placed in the interior of folded blankets and packages of various kinds did not show a temperature capable of killing bacteria after three hours' exposure in a hot-air oven at 133° C. and above.

Moist Heat.—The thermal death-point of bacteria, in the absence of spores, is comparatively low when they are exposed to moist heat. The results of the writer's experiments are given below:

> "In my temperature experiments I have taken great pains to insure the exposure of the test organisms to a uniform temperature, and have adopted ten minutes as the standard time of exposure. The method employed throughout has been as follows: From glass tubing having a diameter of about three-sixteenths of an inch I draw out in the flame of a Bunsen burner a number of capillary tubes, with an expanded extremity which serves as

> an air chamber. A little material from a pure culture of the test organism is drawn into each of these capillary tubes by immersing the open extremity in the culture, after having gently heated the expanded end. The end of the tube is then hermetically sealed by heat These tubes are immersed in a water bath maintained at the desired temperature for the standard time. The bath is kept at a uniform temperature by personal supervision. At the bottom of the vessel is a thick glass plate which prevents the thermometer bulb and capillary tubes, which rest upon it, from being exposed to heat transmitted directly from the bottom of the vessel To further guard against this I am in the habit of applying the flame to the sides of the vessel, and a uniform temperature throughout the bath is maintained by frequent stirring with a glass rod. It is impossible to avoid slight variations, but by keeping my eye upon the thermometer throughout the experiment I have kept these within very narrow limits. . . . No attempt has been made to fix the thermal death-point within narrower limits than 2° C., and in the table the lowest temperature is given which has been found, in the experiments made, to destroy all of the microörganisms in the material subjected to the test. No doubt more extended experiments would result, in some instances, in a reduction of the temperature given as the thermal death-point for a degree or more. But the results as stated are sufficiently accurate for all practical purposes."[1]

The results obtained in these experiments, for non-sporebearing bacteria, are given in the following table. The time of exposure was ten minutes, except for the cholera spirillum and the cheese spirillum of Deneke.

THERMAL DEATH-POINT OF BACTERIA.

	Centigrade.	Fahrenheit.
Spirillum choleræ Asiaticæ	52°	125.6° (4 m.)
Spirillum tyrogenum (cheese spirillum)	52	125.6 (4 m.)
Spirillum Finkler-Prior	50	122.
Bacillus typhi abdominalis	56	138.8
Bacillus of schweine-rothlauf (rouget)	58	136.4
Bacillus murisepticus	58	136.4
Bacillus Neapolitanus (Emmerich's bacillus)	62	143.6
Bacillus cavicida	62	143.6
Bacillus pneumoniæ (Friedländer's)	56	132.8
Bacillus crassus sputigenus	54	129.2
Bacillus pyocyaneus	56	132.8
Bacillus indicus	58	136.4
Bacillus prodigiosus	58	136.4
Bacillus cyanogenus	54	129.2
Bacillus fluorescens	54	129.2
Bacillus acidi lactici	56	132.8
Staphylococcus pyogenes aureus	58	136.4
Staphylococcus pyogenes citreus	62	143.6
Staphylococcus pyogenes albus	62	143.6
Streptococcus pyogenes	54	129.2
Micrococcus tetragenus	58	136.4
Micrococcus Pasteuri	52	125.6
Sarcina lutea	64	147.2
Sarcina aurantiaca	62	143.6

The following determinations of the thermal death-point of pathogenic organisms have been made by the authors named: Bacillus anthracis (Chauveau), 54° C.; Bacillus mallei—the bacillus of glanders—(Löffler), 55° C., Bacillus gallinarum—micrococcus of fowl cholera—(Salmon), 56° C.; Bacillus of diphtheria (Löffler), 60° C.

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[1] Quoted from the Report of the Committee on Disinfectants of the American Public Health Association, pages 136 and 152.

In the writer's experiments the micrococcus of gonorrhœa was apparently killed by exposure for ten minutes to a temperature of 60° C.

"Some gonorrhœal pus from a recent case which had not undergone treatment was collected for me by my friend Dr. Rohé in the capillary glass tubes heretofore described. A microscopical examination of stained cover-glass preparations showed that this pus contained numerous 'gonococci' in the interior of the cells. Two of the capillary tubes were placed in a water bath maintained at 60° C. for ten minutes. The pus was then forced out upon two pledgets of cotton wet with distilled water. Two healthy men had consented to submit to the experiment, and one of these bits of cotton was introduced into the urethra of each and left *in situ* for half an hour. As anticipated, the result was entirely negative. For obvious reasons no control experiment was made to fix the thermal death-point within narrower limits.

"In connection with these experiments upon the thermal death-point of known pathogenic organisms, it is of interest to inquire whether the virulence of infectious material, in which it has not been demonstrated that this virulence is due to a microörganism, is destroyed by a correspondingly low temperature. Evidently, if this proves to be the case, it will be a strong argument in favor of the view that we have to deal with a microörganism in these diseases also. We have experimental proof that a large number of pathogenic organisms are killed by exposure for ten minutes to a temperature of 55° to 60° C. But, so far as I am aware, this low temperature would not be likely to destroy any of the poisonous chemical products which might be supposed to be the cause of infective virulence, leaving aside the fact that such chemical products have no power of self-multiplication, and, therefore, could not be the independent cause of an infectious disease.[1]

"*Vaccine Virus.*—Carstens and Coert have experimented upon the temperature required to destroy the potency of vaccine virus. In a paper read at the International Medical Congress in 1879 they report, as a result of their experiments, that the maximum degree of heat to which fresh vaccine virus can be exposed without losing its virulence probably varies between 52° and 54° C. Fresh animal vaccine heated to 52° C. for thirty minutes does not lose its virulence. Fresh animal vaccine heated to 54.5° for thirty minutes loses its virulence.

"*Rinderpest.*—According to Semmer and Raupach, exposure for ten minutes to a temperature of 55° C. destroys the virulence of the infectious material in this disease.

"*Sheep-pox.*—The authors last mentioned have also found that the same temperature—55° C. for ten minutes—destroys the virulence of the blood of an animal dead from sheep-pox.

"*Hydrophobia.*—Desiring to fix the thermal death-point of the virus of hydrophobia, I obtained, through the kindness of Dr. H. C. Ernst, a rabbit which had been inoculated, by the method of trephining, with material which came originally from Pasteur's laboratory. The rabbit sent me showed the first symptom of paralytic rabies on the eighth day after inoculation. It died on the eleventh day (March 2d, 1887), and I at once proceeded to make the following experiment:

"A portion of the medulla was removed and thoroughly mixed with sterilized water. The milky emulsion was introduced into four capillary tubes, such as had been used in my experiments heretofore recorded. Two of these tubes were then placed for ten minutes in a water bath, the temperature of which was maintained at 60° C. Four rabbits were now inoculated by trephining, two with the material exposed to 60° C. for ten minutes, and two with the same material from the capillary tube not so exposed. The result was as definite and satisfactory as possible. The two control rabbits were taken sick, one on March 10th and one on the 11th ; both died with the characteristic symptoms of paralytic rabies on the third day. The two rabbits inoculated with material exposed to 60° C. remained in perfect health. On the 26th of March one of these rabbits was again inoculated, by trephining, with material from the medulla of a rabbit just dead from hydrophobia. This rabbit died from paralytic rabies on the 8th of April. Its companion remains in perfect health.

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[1] Since this was written Brieger has isolated a toxalbumin from cultures of the diphtheria bacillus which is destroyed by a temperature of 60° C., but resists 50°.

"A second experiment was made in the same way on the 14th of March. Two rabbits were inoculated with material exposed for ten minutes to a temperature of 50° C.; two with material exposed to 55° C.; and two control rabbits with material not so exposed. One of the rabbits inoculated with material exposed to 50° C., and one of the control rabbits, died on the 25th; the other rabbit inoculated with the material exposed to 50°, the other control, and one inoculated with material exposed to 55°, on the 26th. The second rabbit inoculated with material exposed to 55° died five days later with the characteristic symptoms of the disease. These experiments show, then, that the virus of hydrophobia is destroyed by a temperature of 60° C., and that 55° C. fails to destroy it, the time of exposure being ten minutes."[1]

The experimental data given show that the pathogenic bacteria tested and different kinds of virus are all killed by a temperature of 60° C. or below; some, like the cholera spirillum and Micrococcus pneumoniæ crouposæ, failing to grow after exposure to as low a temperature as 52° C. for four minutes. By extending the time a still lower temperature will effect the same result. Thus, according to Chauveau, the anthrax bacillus is killed by twenty minutes' exposure to a temperature of 50° C.; and Brieger sterilizes cultures of the diphtheria bacillus, to obtain the soluble toxalbumin produced in them, by exposure for several hours to 50° C. A temperature of 60° has been found to decompose the toxalbumin. The non-pathogenic bacteria tested have, as a rule, a higher thermal death-point—58° C. for Bacillus prodigiosus, 64° C. for Sarcina lutea, etc.

It is a remarkable fact that certain bacteria not only are not destroyed at higher temperatures than this, but are able to multiply at a temperature of 65° to 70° C. Thus Miquel, in 1881, found in the waters of the Seine a motionless bacillus which grew luxuriantly in bouillon at a temperature of 69° to 70° C. Van Tieghem has also cultivated several different species at about the same temperature, and more recently Globig has obtained from the soil several species which grow at temperatures ranging from 50° to 70° C.

The resisting power of *spores* to heat also varies in different species; but the spores of known pathogenic bacteria are quickly destroyed by a temperature of 100° C. (212° F.). In the writer's experiments the spores of Bacillus anthracis and of Bacillus alvei failed to grow after exposure to a temperature of 100° C. for four minutes, and only a few colonies developed after two minutes' exposure to this temperature. The thermal death-point of spores of the "wurtzel bacillus" and of Bacillus butyricus (of Hueppe) was the same—100° C. for four minutes.

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[1] Report of the Committee on Disinfectants (op. cit.), p. 147.

Schill and Fischer, in 1884, made a number of experiments to determine the thermal death-point of Bacillus tuberculosis. They found that five minutes' exposure to a temperature of 100° C. in steam destroyed the vitality of the bacillus in sputum in five minutes. When the time was reduced to two minutes a negative result from inoculation was obtained in two guinea-pigs, but one inoculated at the same time became tuberculous. My own experiments and those of Yersin, made since, lead me to think that there may have been some cause of error in this experiment of Schill and Fischer, and that the thermal death-point of the spores of Bacillus tuberculosis is considerably below the boiling point of water. I inoculated guinea-pigs with tuberculous sputum subjected for ten minutes to the following temperatures: 50°, 60°, 70°, 80°, 90° C. The animal inoculated with material exposed to 50° died from tuberculosis at the end of seven weeks. None of the others developed tuberculosis.

Yersin exposed an old culture in glycerin bouillon, in which many of the bacilli contained spores—"très nettes"—to the following temperatures: 55°, 60°, 65°, 70°, 75°, 80°, 85°, 90°, 100° C. "At the end of ten days the bacilli heated to 55° gave a culture in glycerin bouillon; those exposed to 60° grew after twenty-two days; none of the bacilli heated above 70° gave any development. This experiment, repeated a great number of times, has always given us the same result." Voelsh, who has studied the same question, reports as the result of his experiments that the tubercle bacillus in sputum was not destroyed by heating to 100° C. Further experiments will be required to reconcile these contradictory results.

While the spores of the pathogenic bacteria mentioned are destroyed by the boiling point of water within a few minutes, certain non-pathogenic species resist this temperature for hours. Thus Globig obtained a bacillus from the soil the spores of which required five and one-half to six hours' exposure to streaming steam for their destruction. These spores survived exposure for three-quarters of an hour in steam under pressure at from 109° to 113° C. They were destroyed, however, by exposure for twenty-five minutes in steam at 113° to 116°, and in two minutes at 127°.

In the practical application of steam for disinfecting purposes it must be remembered that, while steam under pressure is more effective than streaming steam, it is scarcely necessary to give it the preference, in view of the fact that all known pathogenic bacteria and their spores are quickly destroyed by the temperature of boiling water ; and also that superheated steam is less effective than moist steam. When confined steam in pipes is "superheated" it has about the same germicidal power as hot dry air at the same temperature. This is shown by the experiments of Esmarch, who found that anthrax spores were killed in streaming steam in four minutes, but were not killed in the same time by superheated steam at a temperature of 141° C.

Desiccation.—Cultures of bacteria kept in a moist condition retain their vitality for a considerable time, which varies greatly with different species. The writer has found that a culture of the typhoid bacillus preserved in a hermetically sealed glass tube retained its vitality for eighteen months, as did also Bacillus prodigiosus, Bacillus cavicida, and some others. According to Kitasato, the cholera spirillum may be preserved in a moist state for seven months ; other bacteria die out in a month or two, but, as a rule, vitality is preserved for several months at least.

Spores in a desiccated condition preserve their vitality for a great length of time. But desiccation is quickly fatal to some of the pathogenic bacteria, and especially so to the cholera spirillum. Koch, in his earlier experiments, found that his "comma bacillus" did not grow after being dried upon a cover glass for three hours. Kitasato, in experiments made since, found that a bouillon culture dried upon a thin glass cover was incapable of development after three hours' time, but that cultures in nutrient agar or gelatin survived for two days, probably on account of the thicker layer formed and the longer time required for complete desiccation. Pfuhl has found that the typhoid bacillus dried upon a cover glass retains its vitality for eight to ten weeks, and Löffler states that the diphtheria bacillus resists desiccation for four or five months. Cadéac and Malet produced tuberculosis in guinea-pigs by injecting material from the lung of a tuberculous cow which had been kept in the form of a dried powder for nearly five months ; at a later date the virulence was lost.

Light.—Downes and Blunt, in a communication made to the Royal Society of London in 1877, first called attention to the fact that light has an injurious effect upon bacteria, and that cultures may be sterilized by exposure to direct sunlight.

Tyndall, in experiments made in the clear sunlight of the Alps, verified the fact that the development of bacteria was restrained in cultures during their exposure, but failed to obtain evidence that vitality was destroyed.

In 1885 Duclaux took up the subject with pure cultures of various bacteria, and showed that by prolonged exposure to direct sunlight the spores of various bacilli lose their capacity to germinate. About the same time Arloing published his researches upon the influence of light upon the development of anthrax spores. He found that the anthrax bacillus was not restrained in its growth by diffused lamplight, but its growth was retarded by an intense gaslight. Spore formation was more abundant in darkness than in red light, and more abundant in red than in white light. When a screen was interposed between the culture and the source of light, consisting of an aqueous solution of hæmatoglobin, the growth of the bacilli and of spores was much more luxuriant than in white light. In yellow light it was less abundant than in red. The blue and violet rays were still less favorable for the growth of the bacillus and the development of spores. The pathogenic power of cultures was not especially influenced by exposure to white gaslight. In subsequent experiments with sunlight Arloing found that two hours of exposure to the July sun sufficed to destroy the vitality of anthrax spores, but that a considerably longer exposure (twenty-six to thirty hours) was necessary when the spores had been allowed to germinate in a suitable culture medium. Cultures which were not exposed long enough to destroy the vitality of the bacilli were retarded in their growth, and subsequent exposure for a shorter time (nine to ten hours) completely sterilized them. Cultures which were weakened in their reproductive energy by exposure to sunlight were also "attenuated" as to their pathogenic power and could be used as a vaccine in protective inoculations. According to Arloing, the effect produced results from the action of the full sunlight and cannot be obtained by the use of monochromatic light.

The experiments of Strauss seemed to give support to the view advanced by Nocard that in Arloing's experiments spores did not really exhibit a less degree of resisting power than the vegetating bacilli, but that in fact they commenced to vegetate before they were killed. Strauss placed anthrax spores in sterilized distilled water and in bouillon, and found that, under the same conditions of exposure, the bouillon cultures were sterilized in direct sunlight in nine hours, while the spores suspended in distilled water grew when transferred to a suitable medium. This was accounted for on the supposition that the bouillon furnishes the necessary pabulum for the development of the spores and that distilled water does not.

Arloing combats this view and has published additional experiments which seem to disprove it. He placed small flasks containing anthrax spores in bouillon in the direct rays of the sun in February. Some of the flasks were placed upon a block of ice which reduced the temperature to 4° C.; the others were not so placed, and the temperature, in the open air where all were exposed, was 11° C. All of the spores failed to grow after an exposure of four hours. When exposed in water the time of exposure was longer.

Roux has shown that the light also has an effect upon the culture medium, and that sterilized bouillon which has been exposed to direct sunlight for some hours restrains the development of anthrax spores subsequently introduced into it, but not of the growing bacilli. His experiments show that access of oxygen is a necessary factor in the sterilization of cultures by sunlight.

In the experiments of Momont (1892) dry anthrax spores were found to resist the action of light for a long time, but moist spores, freely exposed to the air, failed to grow after forty-four hours' exposure to sunlight. In the absence of spores, anthrax bacilli in a moist condition, when freely exposed to the air, failed to grow after exposure to sunlight for half an hour to two hours ; but in the absence of air the same bacilli were not destroyed at the end of fifty hours' exposure.

Geisler (1892), in experiments made upon the typhoid bacillus, found that all portions of the solar spectrum except the red rays exercised a restraining influence upon the development of this bacillus. The electric light gave a similar result. The most decided effect was produced by rays from the violet end of the spectrum. The restraining influence appears, from the researches of Geisler, not to be due solely to the direct action of light upon the development of the bacilli, but also to changes induced in the gelatin culture medium employed in his experiments.

In his address before the International Medical Congress of Berlin, 1890, Koch states that the tubercle bacillus is killed by the action of direct sunlight in a time varying from a few minutes to several hours, depending upon the thickness of the layer exposed. Diffused daylight also has the same effect, although a considerably longer time of exposure is required—when placed close to a window, from five to seven days.

Dieudonné (1894), in experiments upon Bacillus prodigiosus and Bacillus fluorescens putidus, found that direct sunlight in March, July, and August killed these bacilli in one and one-half hours, in November in two and one-half hours. Diffuse daylight in March and July restrained development after three and one-half hours' exposure (in November four and one-half hours), and completely destroyed vitality in from five to six hours.

Ward's experiments (1892–1894) show that the blue and violet rays have decided germicidal power, while the rays at the red end of the spectrum are comparatively inert. This corresponds with results previously reported by Arloing.

In the writer's experiments on the cholera spirillum (1892) test tubes, containing sterile bouillon inoculated with one or two öse of a pure culture, were sterilized by two hours' exposure to direct sunlight (in December).

Dieudonné (1894) found that the electric arc light destroyed his test organisms (Bacillus prodigiosus and Bacillus fluorescens putidus) in eight hours. The same result was accomplished by the incandescent light in eleven hours.

In view of these facts we may conclude, with Duclaux, that sunlight is one of the most potent and one of the cheapest agents for the destruction of pathogenic bacteria, and that its use for this purpose is to be remembered in making practical hygienic recommendations. The popular idea that the exposure of infected articles of clothing and bedding in the sun is a useful sanitary precaution is fully sustained by the experimental data relating to the action of heat, desiccation, and sunlight.

Electricity.—Cohn and Mendelssohn, in 1879, attempted to determine the effect of the galvanic current upon bacteria. Cultures were placed in U-tubes through which a constant current was passed. A feeble current was found to be without effect. A strong current from two elements, maintained for twenty-four hours, restrained development in the vicinity of the positive pole, but this was probably due to the highly acid reaction which the culture liquid acquired. When a current from five elements was used for twenty-four hours the liquid was sterilized, but this may have been due to the decided changes produced in the chemical composition of the culture liquid rather than to the direct action of the galvanic current.

The same may be said of the similar results obtained in later experiments by Apostoli and Laquerrière, and by Prochownick and Spaeth. The last-mentioned investigators found that the positive pole had a more decided effect than the negative, and that the effect depended upon the intensity and duration of the current. A current of fifty milliampères passed for a quarter of an hour did not kill Staphylococcus pyogenes aureus, but a current of sixty milliampères maintained for the same time did. The spores of Bacillus anthracis required a current of two hundred to two hundred and thirty milliampères during an hour or two. In these experiments the cultures in gelatin were attached to the strips of platinum serving as the two poles, and these were immersed in a solution of sodium chloride. As chlorine was disengaged at the positive pole, the germicidal action is attributed to this gas rather than to the direct action of the current upon the living microörganisms.

The more recent researches of Spilker and Gottstein, made with an induction current from a dynamo machine, are more valuable in estimating the power of this agent to destroy the vitality of bacteria. The current was passed through a spiral wire which was wrapped around a test tube of glass, containing the microörganism to be tested, suspended in distilled water. In a first experiment Bacillus prodigiosus, suspended in sterilized distilled water and contained in test tubes having a capacity of two hundred and fifty cubic centimetres, was subjected to a current having an energy of 2.5 ampères × 1.25 volts for twenty-four hours. The temperature did not go above 30° C. No development occurred when the microörganism tested was subsequently planted in nutrient gelatin. Further experiments gave a similar result. It was found that stronger currents were effective in shorter time; but in no case was sterilization effected in less than an hour.

Pressure.—D'Arsonval and Charrin (1894) submitted a culture of Bacillus pyocyaneus to a pressure of fifty atmospheres, under carbon dioxide. At the end of four hours cultures could still be obtained, but the bacillus had lost its power of pigment production. A few colonies were developed after six hours' exposure to this pressure; but after twenty-four hours no development occurred.

Agitation.—Meltzer (1894) has shown that the vitality of bacteria is destroyed by protracted and violent shaking, which causes a molecular disintegration of the cells.

VII.

ANTISEPTICS AND DISINFECTANTS.

GENERAL ACCOUNT OF THE ACTION OF.

THE term *antiseptic* is used by some authors to designate an agent which destroys the vitality of the microörganisms which produce septic decomposition, and others of the same class. We prefer to restrict the use of the term to those agents which restrain the development of such microörganisms without destroying their vitality. The complete destruction of vitality is effected by *germicides* or disinfectants. Material containing the germs of infectious diseases is infectious material, and we disinfect it by the use of agents which destroy the living disease germs or pathogenic bacteria which give it its infecting power. Such an agent is a disinfectant. But we extend the use of this term to germicides in general—that is, to those agents which kill non-pathogenic bacteria as well as to those which destroy disease germs. All disinfectants are also antiseptics, for agents which destroy the vitality of the bacteria of putrefaction arrest the putrefactive process ; and these agents, in less amount than is required to completely destroy vitality, arrest growth and thus act as antiseptics. But all antiseptics are not germicides. Thus a concentrated solution of salt or of sugar will prevent the putrefactive decomposition of organic material, animal or vegetable ; but these agents do not destroy the vitality of the germs of putrefaction. In a certain degree of concentration they are antiseptics and are largely used for the preservation of meats and vegetables. In the same way many mineral salts in solutions of various strengths act as antiseptics, and some of these in still stronger solutions are disinfectants. Thus mercuric chloride, when introduced into a culture solution in the proportion of 1 : 300,000, will restrain the development of anthrax spores, but to insure the destruction of these spores a solution of 1 : 1,000 must be used. As a rule, the difference between restraining action—antiseptic—and germicidal power—disinfectant—is not so great as this. We give below some recent determinations by Boer which illustrate this point, the test organism being the bacillus of typhoid fever in a culture in bouillon twenty-four hours old :

	Restrains.	Kills.
Hydrochloric acid	1 : 2100	1 : 300
Sulphuric acid	1 : 1550	1 : 500
Silver nitrate	1 : 50000	1 : 4000
Sodium arseniate	1 : 6000	1 : 250
Carbolic acid	1 : 400	1 : 200

Method of Determining Antiseptic Value.—To determine the restraining or antiseptic power of an agent for a particular microörganism, the agent is dissolved in a definite proportion in a suitable culture medium, which is then inoculated with a pure culture of the test organism and placed in favorable circumstances—as to temperature—for its growth. At the same time a control experiment is made by placing another portion of the same culture medium, inoculated with the same microörganism, in the same conditions, but without the addition of the antiseptic agent. If development occurs in the control experiment and not in the culture medium containing the antiseptic, the failure to grow must be attributed to the presence of this agent. Having made a preliminary experiment, we are guided by the result in further experiments to determine the exact amount required to restrain development under the same conditions. Or we may make a series of experiments in the first instance. The problem being, for example, to determine the antiseptic value of carbolic acid for the typhoid bacillus, we may add this agent to a definite amount of bouillon in test tubes in the proportion of 1 : 100, 1 : 200, 1 : 300, 1 : 400, 1 : 500. In experiments with volatile agents the bouillon, in test tubes or small flasks, must be sterilized in advance, and the antiseptic agent introduced by means of a sterilized pipette with great care to prevent the accidental contamination of the nutrient medium. In experiments with non-volatile agents it will be best to sterilize the culture medium after the antiseptic has been added. Next we inoculate the liquid in each flask with a pure culture of the test organism. The flasks are then placed in an incubating oven at 35° to 37° C. At the same time a control, not containing any carbolic acid, is placed in the oven. At the end of twenty-four hours the control will be found to be clouded, showing an abundant multiplication of the bacillus. Taking the result of Boer above given, we would expect to find all of the solutions clear except that containing 1 : 500. This too might remain clear for some days and finally "break down," for experience shows that when we pass the point at which a permanent restraining influence is exerted there may be a temporary restraint or retardation of development. For this reason we must continue the experiment for a considerable time—not less than two weeks. Having found that 1 : 400 and below prevents development, and 1 : 500 does not, we may make further experiments to determine the antiseptic power within narrower limits ; but this is hardly necessary from a practical point of view.

In these experiments the result will be influenced by several circumstances, as follows :

(*a*) *By the composition of the nutrient medium.* This is a very important factor, especially in determining the antiseptic value of certain metallic salts. The presence of a considerable quantity of albumin, for example, reduces greatly the antiseptic power of mercuric chloride, silver nitrate, creolin, etc. The presence of a substance chemically incompatible, as, for example, sodium chloride in testing nitrate of silver, will of course neutralize antiseptic action.

(*b*) *The nature of the test organism.* Within certain limits an antiseptic for one microörganism of this class restrains the development of all, but there are wide differences in the ability of different species to grow in the presence of different chemical agents. Some grow readily in the presence of a considerable amount of free acid, others are restrained by a slightly acid reaction of the medium in which they are placed. The Bacillus acidi lactici, for example, can thrive in the presence of a considerable amount of the acid which is a product of its growth, but there is a limit to its power of developing in the presence of this and other acids. So, too, Micrococcus ureæ, which causes the alkaline fermentation of urine, grows in the presence of a considerable amount of carbonate of ammonia, but is finally restrained in its growth by this alkaline salt. The following determinations by Boer show the difference in the antiseptic power of hydrochloric acid for certain pathogenic bacteria : Bacillus of anthrax (without spores), 1 : 3,400 ; diphtheria bacillus, 1 : 3,400 ; glanders bacillus, 1 : 700 ; typhoid bacillus, 1 : 2,100 ; cholera spirillum, 1 : 5,500. It will be noted that the cholera spirillum is restrained in its growth by about one-eighth the amount of hydrochloric acid which is required to prevent the development of the bacillus of glanders. The typhoid bacillus has a special tolerance for carbolic acid, etc.

(*c*) *The temperature at which the experiment is made.* At the temperature most favorable for growth a greater proportion of the antiseptic agent is required than at unfavorable temperatures—lower or higher.

(*d*) *The restraining influence for spores* is much greater than for the vegetative form of bacteria.

Methods of Determining Germicide Value.—The disinfecting power of a chemical agent is determined by allowing it to act for a given time, in a definite proportion, on a pure culture of a given microörganism, and then testing the question of loss of vitality by culture experiments or by inoculations of infectious disease germs into susceptible animals.

The *test by cultivation* is the most reliable, but in making it several points must be kept in view. Naturally the conditions must be such as are favorable for the growth of the particular microörganism which serves as the test; and we must allow a considerable time for the development of the test organism, for it often happens that its vital activity has been weakened without being completely destroyed, and that growth will occur after an interval of several days, while in the control experiment it has perhaps been seen at the end of twenty-four hours. Another most important point is the fact that some of the disinfecting agent is necessarily carried over with the test organisms when these are transferred to a nutrient medium to ascertain whether they will grow, and this may be in sufficient amount to restrain their development and lead to the mistaken inference that they have been killed. This is especially true of mercuric chloride, which restrains the development of spores in very minute amounts. Spores which have been subjected to its action in comparatively strong solutions, when transferred to a culture medium may fail to grow because of the restraining influence of the mercuric chloride carried over at the same time. For this reason liquid cultures are to be preferred in experiments of this kind. When the test organisms are planted in a solid culture medium the chemical agent is left associated with them; in a liquid culture, on the other hand, it is diluted, and the microörganisms, being distributed through the nutrient medium, have the disinfecting agent washed from their surface. In the case of mercuric chloride, however, the experiments of Geppert show that the agent is so attached to spores which have been subjected to its action that ordinary washing does not suffice. Moreover, spores which have been exposed to the action of mercuric chloride without being killed are restrained in their growth by a much smaller proportion of the corrosive sublimate than is required for spores not so exposed—according to Geppert, by 1 part in 2,000,000. Geppert therefore proposes, in experiments with this agent, to neutralize the mercuric chloride which remains attached to the test organisms by washing these in a solution of ammonium sulphide, by which the sublimate is precipitated as an inert sulphide.

With most agents simple dilution will serve the purpose of preventing an erroneous inference from the restraining influence of the chemical agent being tested. If we carry, by means of a platinum loop, one or two öse into five to ten cubic centimetres of bouillon, the dilution will usually be beyond the restraining influence of the germicidal agent ; but we may carry the dilution still further, to be on the side of safety, by inoculating a second tube containing the same amount of sterile bouillon from the first, carrying over in the same way one or two öse. We will still be very sure to have a considerable number of the microörganisms to test the question of the destruction of vitality. Instead of bouillon we may use liquefied flesh-peptone-gelatin, which gives us the same advantage as to dilution of the disinfecting agent ; and after inoculating two tubes as above indicated, we may make Esmarch roll tubes by turning them upon a block of ice. The development of colonies will show that there was a failure to disinfect ; their absence, after a proper interval, will be evidence of the germicidal action of the agent employed.

Koch's Method.—In 1881 Koch published his extended experiments made to determine the germicidal power of various chemical agents as tested upon anthrax spores. His method consisted in exposing silk threads, to which the dried spores were attached, in a solution of the disinfecting agent, and at intervals transferring one of these threads to a solid culture medium. The precaution was taken to wash the thread in distilled water when the agent tested was supposed to be likely to restrain development. In these experiments a standard solution of the disinfecting agent was used, and the time of exposure was varied from a few hours to many days.

The Writer's Method.—In the writer's experiments, made in 1880 and subsequently, a different method has been adopted. The time has been constant—usually two hours—and the object has been to find the minimum amount of various chemical agents which would destroy the test organisms in this time ; and instead of subjecting a few of the test organisms attached to a silk thread to the action of the disinfecting agent, a certain quantity of a recent culture—usually five cubic centimetres—has been mixed with an equal quantity of a standard solution of the germicidal agent. Thus five cubic centimetres of a 1 : 200 solution of carbolic acid would be added to five cubic centimetres of a recent culture of the typhoid bacillus, for example, and after two hours' contact one or two öse would be introduced into a suitable nutrient medium to test the question of disinfection. In the case given the result obtained would be set down as the action of a solution of carbolic acid in the proportion of 1 : 400, for the 1 : 200 solution was diluted by the addition of an equal quantity of the culture.

Other experimenters have adopted still a different method. Instead of using a considerable and definite quantity of a culture containing the test organism, they introduce one or two öse from such a culture into a solution containing a given proportion of the disinfectant ; then after exposure for a given time the nutrient medium is inoculated.

These different methods give results which cannot be directly compared one with another, for to obtain corresponding results we must have identical conditions.

Test by Inoculation into Susceptible Animals.—In testing the action of disinfectants upon anthrax spores and other infectious disease germs, we may inoculate the microörganisms, after exposure to the disinfectant, into a susceptible animal. This method was adopted by the writer in a series of experiments in 1881, but he has not since employed it, for reasons set forth in his paper giving an account of these experiments.

"*First.* The test organism may be modified as regards reproductive activity without being killed; and in this case a modified form of disease may result from the inoculation, of so mild a character as to escape observation. *Second.* An animal which has suffered this modified form of the disease enjoys protection, more or less perfect, from future attacks, and if used for a subsequent experiment may, by its immunity from the effects of the pathogenic test organism, give rise to the mistaken assumption that this had been destroyed by the action of the germicidal agent to which it had been subjected."[1]

In experiments to determine the value of an agent as a disinfectant, no matter by what method, the following conditions, which influence the result, should be kept in view :

(*a*) *The difference in vital resisting power of different species of bacteria.* As a rule, the pathogenic species have rather less resisting power than the common saprophytes, and the micrococci have greater resisting power than many of the bacilli. The difference in the vital resisting power of some of the best known pathogenic species is shown in the following table, which we have made up from determinations made by Boer—cultures in bouillon twenty-four hours old ; time of exposure, two hours.

	Hydrochloric Acid.	Caustic Soda.	Chloride of Gold and Sodium.	Nitrate of Silver.	Carbolic Acid.
Anthrax bacillus.......	1 : 1100	1 : 450	1 : 8000	1 : 20000	1 : 300
Diphtheria bacillus.....	1 : 700	1 : 300	1 : 1000	1 : 2500	1 : 300
Glanders bacillus	1 : 200	1 : 150	1 : 400	1 : 4000	1 : 300
Typhoid bacillus	1 : 300	1 : 190	1 : 500	1 : 4000	1 : 200
Cholera spirillum......	1 : 1350	1 : 150	1 : 1000	1 : 4000	1 : 400

(*b*) *The presence or absence of spores.* The reproductive elements known as spores have a far greater resisting power to chemical agents, as well as to heat, than have the vegetative cells. In practical disinfection, therefore, it is important to know what disease germs form spores and what do not. The following are known to form spores: The bacillus of anthrax, the bacillus of tetanus, the bacillus of malignant œdema, the bacillus of symptomatic anthrax, the bacillus of foul brood (infectious disease of bees). The following, so far as is known, do not form spores: The pus cocci (Staphylococcus pyogenes albus, aureus, and citreus, and Streptococcus pyogenes), the micrococcus of pneumonia, the bacillus of typhoid fever, the bacillus of glanders, the bacillus of diphtheria, the spirillum of cholera, the spirillum of relapsing fever.

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[1] Quoted from article on "Germicides and Disinfectants," in "Bacteria," p. 212.

Many agents which kill the growing bacteria are incapable of destroying the vitality of spores, and others only do so in much stronger solutions or after a long exposure to their action.

(*c*) *The number of bacteria to be destroyed.* This is an essential factor which has often been overlooked by those making experiments. To destroy the bacteria carried over to five cubic centimetres of distilled water by means of a platinum loop, is a very different matter from destroying the immensely greater number in five cubic centimetres of a recent bouillon culture.

(*d*) *The nature and quantity of associated material.* The oxidizing disinfectants, like permanganate of potash and chloride of lime, not only act upon the bacteria, destroying them by oxidation, but upon all organic matter with which they come in contact, and at the same time the disinfecting agent is destroyed in the chemical reaction, which is a quantitative one. The presence, therefore, of organic material in association with the bacteria is an important factor, and if this is in excess the disinfectant may be neutralized before the living bacteria are destroyed. Other substances which precipitate the disinfecting agent in an insoluble form, or decompose it, must of course have the same effect. Thus the presence of sodium chloride in a culture medium would be an important circumstance if nitrate of silver was the agent being tested, as the insoluble chloride would be precipitated. And in the case of mercuric chloride and certain other metallic salts the presence of albumin very materially influences the result. Van Ermengem states that the cholera spirillum in bouillon is destroyed in half an hour by mercuric chloride in the proportion of 1:60,000, while in blood serum 1:800 was required to destroy it in the same time.

(*e*) *The time of exposure* is also an important factor. Some agents act very promptly, while others require a considerable time to effect the destruction of bacteria exposed to their action. Thus a solution of chloride of lime containing 0.12 per cent destroys the typhoid bacillus and the cholera spirillum in five minutes, and the anthrax bacillus in one minute (Nissen). On the other hand, quicklime (milk of lime) requires a contact of several hours to insure the destruction of pathogenic bacteria.

(*f*) *The temperature at which the exposure is made* has a material influence upon the result. This is shown by the experiments of Henle and of Nocht. As a general rule germicidal activity increases in direct proportion to the increase in temperature from 20° C. upward.

(*g*) *The degree of dilution of the disinfecting agent* is also a matter of importance. This is especially true of solutions of acids and alkalies. When a silk thread to which bacteria are attached is suspended in an acid solution the essential point is the degree of acidity, and not the quantity of acid in the entire solution. But if a solution of permanganate of potash, or any other active oxidizing agent, is used, the principal question is not the degree of dilution, but the amount of the disinfecting agent present in the solution used. A grain of potassium permanganate dissolved in two fluidounces of distilled water would probably kill just as many bacteria as if it were dissolved in half a fluidounce, although the time required for disinfection might be longer.

From what has been said it is evident that the simple statement that a certain agent is a germicide in a certain proportion has but little scientific value, unless we are made acquainted with the conditions under which its germicidal action has been tested.

VIII.

ACTION OF GASES AND OF THE HALOID ELEMENTS UPON BACTERIA.

Oxygen.—Free oxygen is essential for the development of a large number of species of bacteria—aërobics; and it completely prevents the growth of others—anaërobics. Many bacteria, even when freely exposed in a desiccated condition to the action of atmospheric oxygen, retain their vitality for a long time. The gradual loss of pathogenic power which Pasteur has shown occurs in cultures of the anthrax bacillus and the micrococcus of fowl cholera, is ascribed by him to exposure to oxygen, and as proof of this he states that cultures kept in hermetically sealed tubes do not lose their virulence in the same degree. But other circumstances may influence the result. Thus some of the products of growth which accumulate in culture fluids have an injurious effect upon the vitality of the bacteria which produced them, and in time may cause a complete destruction of vitality. In cultures exposed to the air these products would be in a more concentrated solution from the gradual evaporation of the culture liquid. It must also be remembered that light in the presence of oxygen is a germicidal agent.

The experiments of Fränkel show that the aërobic bacteria grow abundantly in the presence of pure oxygen, and some species even more so than in ordinary air. Micrococcus prodigiosus, however, appeared to be unfavorably affected by pure oxygen, inasmuch as it did not produce pigment so readily as when cultivated in ordinary air.

Nascent oxygen is a very potent germicidal agent, as will be seen in our account of such oxidizing disinfectants as potassium permanganate and the hypochlorite of lime.

Ozone.—It was formerly supposed that ozone would prove to be a most valuable agent for disinfecting purposes; but recent experiments show that it is not so active a germicide as was anticipated, and that from a practical point of view it has comparatively little value.

Lukaschewitsch found that one gramme in the space of a cubic metre failed to kill anthrax spores in twenty-four hours. The cholera spirillum in a moist state was killed in this time by the same amount, but fifteen hours' exposure failed to destroy it. Ozone for these experiments was developed by means of electricity.

Wyssokowicz found that the presence of ozone in a culture medium restrained the development of the anthrax bacillus, the bacillus of typhoid fever, and others tested, but concludes that this is rather due to the oxidation of bases contained in the nutrient medium than to a direct action upon the pathogenic bacteria.

Sonntag, in his carefully conducted experiments, in which a current of ozonized air was made to pass over silk threads to which were attached anthrax spores, had an entirely negative result. The anthrax bacillus from the spleen of a mouse, and free from spores, was then tested, also with a negative result, even after exposure to the ozonized air for twenty minutes at a time on four successive days. In another experiment several test organisms (Bacillus anthracis, Bacillus pneumoniæ of Friedländer, Staphylococcus pyogenes aureus, Staphylococcus pyogenes albus, Bacillus murisepticus, Bacillus crassus sputigenus) were exposed on silk threads for twenty-four hours in an atmosphere containing 4.1 milligrammes of ozone to the litre of air (0.19 volumes per cent). The result was entirely negative. When the amount was increased to 13.53 milligrammes per litre the anthrax bacillus and Staphylococcus pyogenes albus failed to grow after twenty-four hours' exposure. The conclusion reached by Nissen, from his own experiments and a careful consideration of those previously made by others, is that ozone is of no practical value as a germicide in therapeutics or disinfection.

Hydrogen.—This gas has no injurious effect upon bacteria, as is shown by the fact that the anaërobic and facultative anaërobic species grow readily in an atmosphere of pure hydrogen.

Hydrogen peroxide in solution in water is a valuable antiseptic and deodorant, but its value as a germicide has been very much overestimated. Miquel, in his experiments to determine the antiseptic value of various agents, places H_2O_2 third in the list of "substances eminently antiseptic," and states that it prevents the development of the bacteria of putrefaction in the proportion of 1:20,000.

In the writer's experiments (1885) a solution was used which contained at first 4.8 per cent of H_2O_2, and five per cent of sulphuric acid which was added by the chemist who prepared the solution, to prevent loss of the hydrogen peroxide. At the end of a month the amount of H_2O_2 was again estimated, and found to be 3.98 per cent. Five weeks later the proportion was 2.4 per cent. Tested upon "broken-down" beef tea, this solution was found to destroy the vitality of the bacteria of putrefaction contained in it, in two hours' time, in the proportion of thirty per cent (about 1.2 per cent of H_2O_2). Anthrax spores were killed in the same time by a twenty-per-cent solution (0.8 per cent H_2O_2). Tested upon a pure culture of pus cocci, it was active in the proportion of ten per cent (0.4 per cent of

H_2O_2); a solution containing 0.24 per cent of H_2O_2 failed to kill pus cocci. But the solution used in these experiments contained also five per cent of sulphuric acid, which by itself kills micrococci in the proportion of 1:200. My conclusion was that, unless the chemists can furnish more concentrated solutions which will keep better than that with which I experimented, we are not likely to derive any practical benefit from the use of hydrogen peroxide as a disinfectant.

Altehofer more recently has experimented with a solution containing 9.7 per cent of H_2O_2, and reports the following results: He added to ninety-eight cubic centimetres of hydrant water two cubic centimetres of a bouillon culture of the typhoid bacillus, and to this was added sufficient of his aqueous solution of H_2O_2 to make the proportion present 1:1,000. At the end of twenty-four hours the bacillus was proved by culture experiments to be killed. Water containing the cholera spirillum, treated in the same way, was not entirely sterilized, as a few colonies developed in Esmarch roll tubes; but the general result of his experiments was that the ordinary water bacteria, and the pathogenic bacteria named (cholera, typhoid) when suspended in water, required for their destruction exposure for twenty-four hours in a solution containing one part of H_2O_2 in one thousand of water.

Carbon Dioxide.—The experiments of Fränkel show that certain bacteria grow in an atmosphere of CO_2 as well as in the air; among these are the bacillus of typhoid fever and the pneumonia bacillus of Friedländer. Other species are slightly restricted in their growth, *e.g.* Bacillus prodigiosus, Proteus vulgaris. Still others grow only when the temperature is elevated, including the pus cocci and the bacillus of swine pest. Most of the saprophytic bacteria failed to grow in an atmosphere of CO_2, although their vitality was not destroyed by it. Certain pathogenic species were, however, killed by the action of this gas, among others the cholera spirillum, Bacillus anthracis, and Staphylococcus pyogenes aureus.

Leone and Hochstetter had previously reported that certain bacteria are injuriously affected by CO_2. Fränkel also found that the growth of strictly anaërobic species was restricted in an atmosphere of carbon dioxide. The aërobic species which failed to grow in pure CO_2 grew abundantly when a little atmospheric oxygen was admitted. In the experiments of Frankland the cholera spirillum and the Finkler-Prior spirillum failed to develop in an atmosphere of CO_2, and at the end of eight days were no longer capable of growth when the carbon dioxide was replaced with atmospheric air.

Carbonic Oxide.—Frankland's experiments show that an atmosphere of this gas is not favorable to the growth of the cholera spirillum or of the Finkler-Prior spirillum, although it did not entirely prevent development, and after seven days' exposure the spirilla were not all killed, although a comparatively small number of colonies developed. Bacillus pyocyaneus failed to grow in an atmosphere of CO, but when air was admitted, at the end of seven or eight days, abundant development occurred.

Methane, CH_4.—We have no exact experiments to determine the action of marsh gas in a pure state on bacteria, but the experiments of Kladakis upon illuminating gas may be taken as representing approximately what might be expected from exposure in pure CH_4. An analysis of the gas used in his experiments showed it to contain 37.97 per cent of hydrogen, 39.37 per cent of methane (CH_4), 9.99 per cent of nitrogen, 4.29 per cent of ethene (C_2H_4), 3.97 per cent of carbonic oxide (CO), 0.61 per cent of oxygen, and 0.41 per cent of carbon dioxide. As hydrogen and nitrogen are neutral, and carbonic oxide is shown by the experiments of Frankland not to act as a germicide after several days' exposure to its action, the positive results obtained in the experiments of Kladakis may be ascribed to the presence of CH_4 (39.37 per cent) or of C_2H_4 (4.29 per cent), or of both together.

A large number of microörganisms were tested, and among these Proteus vulgaris alone grew in an atmosphere of illuminating gas. The others not only failed to grow in such an atmosphere, but were destroyed by it. Cultures of Bacillus anthracis, Staphylococcus pyogenes aureus, and Spirillum choleræ Asiaticæ were sterilized in half an hour by the action of this gas. The gas was also found to be unsuitable for anaërobic cultures.

Nitrous Oxide, N_2O.—The experiments of Frankland, made upon the cholera spirillum, the spirillum of Finkler-Prior, and the bacillus of green pus, gave results similar to those obtained with CO, viz., seven days' exposure in an atmosphere of this gas failed to destroy the test organisms, but completely restrained the growth of Bacillus pyocyaneus and interfered materially with the development of the two species of spirillum without entirely preventing it.

Nitrogen Dioxide, NO.—Frankland found that his test organisms were quickly killed by this gas (Bacillus pyocyaneus, Spirillum choleræ Asiaticæ, Spirillum Finkler-Prior).

Hydrosulphuric Acid, H_2S.—In the experiments of Frankland this gas proved to be quickly fatal to the bacteria tested (Bacillus pyocyaneus, Spirillum choleræ Asiaticæ, Spirillum Finkler-Prior). On the other hand, Grauer found that this gas did not exercise any injurious influence upon the tubercle bacillus, the bacillus of anthrax, the typhoid bacillus, or the cholera spirillum, after the exposure of these microörganisms in a current of the gas for an hour.

It has been shown by the experiments of Holschewnikoff and others that certain species of bacteria cause an abundant evolution of H_2S as a result of their development in an albuminous medium (Bacillus sulfureus and Proteus sulfureus).

Sulphur Dioxide, SO_2.—Very numerous experiments have been made with this gas, owing to the fact that it has been extensively used in various parts of the world for the disinfection of hospitals, ships, apartments, clothing, etc.

In the writer's experiments, made in 1880, dry vaccine virus on ivory points was disinfected by exposure for twelve hours in an atmosphere containing one volume per cent of this gas, and liquid virus, exposed in a watch glass, by one-third of this amount. Subsequent experiments (1885) showed that pus micrococci were killed by exposure for eighteen hours in a dry atmosphere containing twenty volumes per cent of SO_2, but that four volumes per cent failed. In the presence of moisture this gas has considerably greater germicidal power than this, owing, no doubt, to the formation of the more active agent, sulphurous acid (H_2SO_3). But in a pure state anhydrous sulphur dioxide does not destroy spores. The writer has shown that the spores of Bacillus anthracis and Bacillus subtilis are not killed by contact for some time with liquid SO_2 (liquefied by pressure). Koch exposed various species of spore-bearing bacilli in a disinfection chamber for ninety-six hours, the amount of SO_2 at the outset of the experiment being 6.13 volumes per cent, and at the end 3.3 per cent. The result was entirely negative.

But in the absence of spores the anthrax bacillus, in a moist condition, attached to silk threads, was destroyed in thirty minutes in an atmosphere containing one volume per cent.

In another of Koch's experiments the amount of SO_2 in the disinfection chamber was at the outset 0.84 per cent, and at the end of twenty-four hours 0.55 per cent. An exposure of one hour in this atmosphere killed anthrax bacilli attached to silk threads, in a moist condition; but four hours' exposure failed to kill Bacillus prodigiosus growing on potato, while twenty-four hours' exposure was successful. A similar result was obtained with Bacillus pyocyaneus.

Thinot, as a result of experiments made in 1890, arrives at the conclusion that the specific germs of tuberculosis, glanders, farcy of cattle, typhoid fever, cholera, and diphtheria are destroyed by twenty-four hours' exposure in an atmosphere containing SO_2 developed by the combustion of sixty grains of sulphur per cubic metre. This amount corresponds closely with that fixed by the Committee on Disinfectants of the American Public Health Association on the experimental evidence obtained by the writer in 1885. But the committee insisted upon the presence of moisture and made the time of exposure twelve hours—"exposure for twelve hours to an atmosphere containing at least four volumes per cent of this gas in the presence of moisture."

Chlorine.—The haloid elements are active germicidal agents, especially chlorine on account of its affinity for hydrogen, and the consequent release of nascent oxygen when it comes in contact with microörganisms in a moist condition. And for the same reason this agent is a much more active germicide in the presence of moisture than in a dry condition. The experiments of Fischer and Proskauer showed that when dried anthrax spores were exposed for an hour in an atmosphere containing 44.7 per cent of dry chlorine they were not destroyed; but if the spores were previously moistened and were exposed in a moist atmosphere for the same time, four per cent was effective, and when the time was extended to three hours one per cent destroyed their vitality. The anthrax bacillus, in the absence of spores, was killed by exposure in a moist atmosphere containing 1 part to 2,500, the time of exposure being twenty-four hours, and the same amount was effective for Micrococcus tetragenus; the streptococcus of erysipelas and the micrococcus of fowl cholera were killed in three hours by 1 : 2,500, and in twenty-four hours by 1 : 25,000. The bacillus of mouse septicæmia and the tubercle bacillus were killed in one hour by 1 : 200.

In the writer's experiments (1880) four children were vaccinated with virus from ivory points which had been exposed for six hours in an atmosphere containing one-half per cent of chlorine; also with four points, from the same lot, not disinfected. Vaccination was unsuccessful in every case with the disinfected points, and successful with those not disinfected. Koch found that anthrax spores failed to grow after twenty-four hours' exposure in chlorine water. In the experiments of De la Croix to determine the antiseptic power of this agent, it was found that when present in unboiled beef infusion in the proportion of 1 : 15,600 no development of bacteria occurred. Miquel gives the antiseptic value of chlorine as 1 : 4,000.

Chloroform.—Immersion for one hundred days in chloroform does not destroy the vitality of anthrax spores (Koch). This agent is without effect on the virus of symptomatic anthrax (Arloing, Cornevin, and Thomas). Salkowski found that the anthrax bacillus in the absence of spores, and the cholera spirillum, were killed by being immersed in chloroform water for half an hour. Kirchner reports still more favorable results. In his experiments a one-per-cent solution killed the cholera spirillum in less than a minute, and a one-quarter-per-cent solution in an hour. But the typhoid bacillus required at least one-half per cent acting for an hour.

Iodine.—In the writer's experiments (1880) iodine in aqueous solution with potassium iodide was found to be fatal to Micrococcus pneumoniæ crouposæ in the proportion of 1 : 1,000, and to the staphylococci of pus in 1 : 500—time of exposure two hours. Iodine water was found by Koch to destroy the vitality of anthrax spores in twenty-four hours, but a two-per-cent solution in alcohol failed to destroy anthrax spores in forty-eight hours. In the experiments of Schill and Fischer twenty hours' contact with a solution of the strength of 1 : 500 failed to destroy the virulence of tuberculous sputum, as tested by inoculation experiments. The antiseptic value of iodine is given by Miquel as 1 : 4,000.

Bromine.—Fischer and Proskauer have studied the action of bromine vapor upon various microörganisms. They found that exposure for three hours in a dry atmosphere to three per cent does not destroy the tubercle bacillus in sputum or the spores of anthrax. But when the atmosphere is saturated with moisture 1 : 500 is effective ; and when the time of exposure was extended to twenty-four hours, 1 : 3,500. A two-per-cent solution destroys the vitality of anthrax spores in twenty-four hours (Koch). Bromine vapor is an active agent for the destruction of the virus of symptomatic anthrax (Arloing, Cornevin, and Thomas). Miquel gives the antiseptic value of bromine as 1 : 1,666, which is considerably below that of chlorine and iodine.

Iodine Trichloride.—According to Behring, we possess in this agent a disinfectant which possesses the potency of free chlorine and iodine without having their disadvantages. As prepared by O. Riedel it is a yellowish-red powder of penetrating odor. It remains unchanged for weeks in concentrated aqueous solution (five per cent). A one-per-cent solution destroys anthrax spores suspended in water almost instantly, and a 0.2-per-cent solution within a few minutes. Anthrax spores in blood serum are killed by a one-per-cent solution in forty minutes (Behring). Langenbuch found that a solution of 1 : 1,000 kills spores in a short time, and that when added to nutrient gelatin in the proportion of 1 : 1,200 it restrains the development of bacteria.

Iodoform.—Numerous experiments have been made with this agent, which show that it has little, if any, germicidal power ; but it acts to some extent as an antiseptic. Tilanus reports that the tubercle bacillus will not grow in glycerin-agar cultures to which a small quantity of iodoform has been added, and that a pure culture of the tubercle bacillus was not killed in six days by exposure to iodoform vapor, but that after six weeks' exposure it failed to grow. The experiments of Neisser and of Buchner show that while most bacteria are not injuriously affected by exposure to iodoform vapor, the cholera spirillum and the Finkler-Prior spirillum are restrained in their growth by such exposure. When plate cultures of the cholera spirillum were placed under a bell jar beside iodoform powder no development occurred, but when they were removed colonies developed, showing that the spirilla were not killed.

Iodoform Ether, according to Yersin, is fatal to the tubercle bacillus in one-per-cent solution in five minutes. Cadéac and Meunier found that a saturated solution required thirty-six hours to kill the bacillus of typhoid fever.

Iodol.—In experiments made by the writer (1885) this agent was found to be without germicidal power. Riedlin found it without any action, even upon the cholera spirillum.

Hydrofluoric Acid, HFl.—From a series of experiments made with this gas, Grancher and Chàutard arrive at the conclusion that "the direct and prolonged action of hydrofluoric acid upon the tubercle bacillus diminishes its virulence but does not kill it."

Sozoiodol Acid, according to Dräer, is a phenol, in which two atoms of hydrogen are replaced by two of iodine and one atom by the group HSO_3. This acid and its salts with soda, potash, zinc, and mercury have been tested by the author named. The acid and its salt with mercury were found to destroy the cholera spirillum in two hours' time in two-per-cent solution. A two-per-cent solution of phenol would have accomplished the same result and in less time. *Tribromphenol*, according to Dräer, is less active than sozoiodol acid; and it appears from the experimental evidence on record that combinations of iodine, chlorine, or bromine with phenol are less active that the haloid elements alone. According to Karpow (1893) *monochlorphenol*, tested upon anthrax spores attached to silk threads, proved to be decidedly more active than phenol.

Nosophen (tetraiodphenolphthalein), according to Lieven (1895) contains sixty-one per cent of iodine. It is entirely insoluble in water. When added to nutrient gelatin in the proportion of one-quarter per cent it prevented the development of the anthrax bacillus and of Staphylococcus aureus, but failed to prevent the development of Bacillus pyocyaneus (Lieven).

IX.

ACTION OF ACIDS AND ALKALIES.

Sulphuric Acid, H_2SO_4.—The experiments of Koch (1881) showed that anthrax spores were still capable of growing after exposure in a one-per-cent solution of sulphuric acid for twenty days. In the writer's experiments (1885) a four-per-cent solution failed to destroy the spores of Bacillus subtilis in four hours, and an eight-per-cent solution was found to be required for the sterilization of culture fluids containing spores; but the multiplication of the bacteria of putrefaction was prevented by the presence of this acid in a culture solution in the proportion of 1 : 800. Pus micrococci were destroyed by exposure for two hours in a solution containing 1 : 200.

The experiments of Boer show that there is a considerable difference in the resisting power of different pathogenic bacteria. The time of exposure being two hours, cultures in bouillon twenty-four hours old gave the following results:

	Restrains development.	Destroys vitality.
Anthrax bacillus	1 : 2550	1 : 1300
Diphtheria bacillus	1 : 2050	1 : 500
Glanders bacillus	1 : 750	1 : 200
Typhoid bacillus	1 : 1550	1 : 500
Cholera spirillum	1 : 7000	1 : 1300

Leitz, in his studies relating to the bacillus of typhoid fever, reports the following results: The dejections of typhoid patients, mixed with an equal proportion of the disinfecting solution, were sterilized by a five-per-cent solution of sulphuric acid in three days. A pure culture was sterilized in fifteen minutes by two per cent, and in five minutes by five per cent.

Sulphurous Acid, H_2SO_3.—In the writer's experiments (1885) micrococci were destroyed in two hours by 1 : 2,000 by weight of SO_2 added to water. Kitasato found that solutions of sulphurous acid in the proportion of 0.28 per cent killed the typhoid bacillus, and 0.148 per cent the cholera spirillum. De la Croix found that one gramme of SO_2 added to two thousand of bouillon prevents the development of putrefactive bacteria and after a time destroys the vitality of these bacteria. The writer found that pus cocci failed to grow in a culture solution containing one part of SO_2 in five thousand of water.

Nitric Acid, HNO_3.—In the writer's experiments an eight-per-cent solution which contained 0.819 gramme of HNO_3 in each cubic centimetre sterilized broken-down beef tea containing spores, and five per cent failed to do so. Kitasato, in experiments upon the cholera spirillum and typhoid bacillus, obtained results corresponding with those obtained with hydrochloric acid—0.2 per cent destroyed vitality at the end of four or five hours. In these experiments the acid used contained 0.35 gramme HNO_3 in one cubic centimetre.

Nitrous Acid.—In the writer's experiments on vaccine virus (1880) exposure for six hours in an atmosphere containing one per cent of nitrous acid destroyed the virulence of dried virus upon ivory points.

Hydrochloric Acid, HCl.—Anthrax spores are destroyed in ten days by a two-per-cent solution, but not in five days (Koch). Tested upon broken-down beef tea containing spores of Bacillus subtilis, it was effective in two hours in the proportion of fifteen per cent, but failed in ten per cent (Sternberg). In the experiments of Kitasato this acid destroyed the typhoid bacillus in five hours in the proportion of 0.2 per cent, and the cholera spirillum in 0.132 per cent—the acid used contained 0.26 gramme HCl in one cubic centimetre. We give the more recent determinations of Boer in tabular form. Its germicidal power was tested upon bouillon cultures which had been kept for twenty-four hours in an incubating oven; time of exposure to the action of the acid solution, two hours.

	Restrains development.	Destroys vitality.
Anthrax bacillus	1 : 3400	1 : 1100
Diphtheria bacillus	1 : 3400	1 : 700
Glanders bacillus	1 : 700	1 : 200
Typhoid bacillus	1 : 2100	1 : 300
Cholera spirillum	1 : 5500	1 : 1350

Chromic Acid.—In Koch's experiments a one-per-cent solution destroyed anthrax spores in from one to two days. In the proportion of 1 : 5,000 it prevents the development of putrefactive bacteria (Miquel).

Osmic Acid.—A solution of one per cent kills anthrax spores in twenty-four hours (Koch). It is an antiseptic in the proportion of 1 : 6,666 (Miquel).

Phosphoric Acid.—Exposure for four or five hours to a solution containing 0.3 per cent destroys the typhoid bacillus, and 0.183 per cent the cholera spirillum (Kitasato). The acid used contained 0.152 gramme H_3PO_4 in one cubic centimetre.

Acetic Acid.—A five-per-cent solution failed to kill anthrax spores after five days' exposure (Koch). In Abbott's experiments glacial acetic acid in fifty-per-cent solution failed in two hours to kill anthrax spores, but micrococci were killed by two hours' exposure to a one-per-cent solution. A solution of 1 : 300 of glacial acetic acid destroys the cholera spirillum in half an hour (Van Ermengem). In the proportion of 0.25 per cent it restrains the growth of the typhoid bacillus, and 0.3 per cent destroys its vitality after five hours' exposure; the cholera spirillum fails to grow in presence of 0.132 per cent and is destroyed by 0.2 per cent (Kitasato).

Lactic Acid.—The bacillus of typhoid fever is killed in five hours by a solution containing 0.4 per cent, the cholera spirillum by 0.3 per cent (Kitasato).

Citric Acid.—The bacillus of typhoid fever is killed in five hours by 0.43 per cent, the cholera spirillum by 0.3 per cent (Kitasato). The cholera spirillum is killed in half an hour by 1 : 200 (Van Ermengem).

Oxalic Acid.—The typhoid bacillus requires a solution of 0.36 per cent, the cholera spirillum one of 0.28 per cent, to destroy vitality in five hours (Kitasato).

Boracic Acid.—In the writer's experiments (1883) a saturated solution failed to kill pus cocci in two hours. A five-per-cent solution failed to destroy anthrax spores in five days (Koch). The typhoid bacillus is killed in five hours by 2.7 per cent, the cholera spirillum by 1.5 per cent (Kitasato). According to Arloing, Cornevin, and Thomas, the fresh virus of symptomatic anthrax requires exposure to a twenty-per-cent solution for forty-eight hours for the destruction of vitality. Boracic acid acts as an antiseptic in the proportion of 1 : 143 (Miquel).

Salicylic Acid.—In the writer's experiments this agent was dissolved by the addition of sodium biborate, which by itself has no germicidal power. A two-per-cent solution was found to destroy pus cocci in two hours. Dissolved in oil or in alcohol a five-per-cent solution does not destroy anthrax spores (Koch). Micrococci are destroyed by solutions containing 1 : 400 (Abbott). The typhoid bacillus is killed in five hours by 1.6 per cent, the cholera spirillum by 1.3 per cent (Kitasato). A one-per-cent solution destroys Micrococcus Pasteuri in half an hour (Sternberg). It is an antiseptic in the proportion of 1 : 1,000 (Miquel). A solution of 2.5 per cent kills the tubercle bacillus in six hours (Yersin). In the proportion of 1 : 300 it destroys the cholera spirillum in half an hour (Van Ermengem).

Benzoic Acid.—According to Miquel, this acid restrains the development of putrefactive bacteria when present in bouillon in the proportion of 1:909. In the proportion of 1 : 2,000 it retards the development of anthrax spores (Koch).

Formic Acid.—The typhoid bacillus is restrained in its growth by 0.25 per cent, and is killed in five hours by 0.35 per cent, the cholera spirillum by 0.22 per cent (Kitasato).

Tannic Acid.—A solution of one per cent kills Micrococcus Pasteuri in the blood of a rabbit in half an hour (Sternberg). A five-per-cent solution failed in ten days to destroy anthrax spores (Koch). A twenty-per-cent solution failed in two hours to destroy the vitality of spores of the anthrax bacillus or of Bacillus subtilis (Abbott). Micrococci are destroyed by 1 : 400, and 1 : 800 failed (Abbott). A twenty-per-cent solution has no effect upon the virus of symptomatic anthrax (Arloing, Cornevin, and Thomas). A solution of 1.66 per cent kills the typhoid bacillus in five hours, and 1.5 per cent the cholera bacillus in the same time (Kitasato). It restrains the development of putrefactive bacteria in the proportion of 1 : 207 (Miquel).

Tartaric Acid.—A twenty-per-cent solution of this acid fails, after two hours' exposure, to destroy the spores of Bacillus anthracis or Bacillus subtilis. Micrococci are killed by two hours' exposure in a solution containing 1 : 400 (Abbott).

Malic Acid.—This was found by Kitasato to correspond with citric acid in its germicidal power.

Valerianic Acid.—A five-per-cent solution in ether failed in five days to destroy anthrax spores (Koch).

Oleic Acid.—A solution of five per cent in ether does not destroy anthrax spores in five days (Koch).

Thymic Acid.—In the proportion of 1 : 500 this acid prevents the putrefactive decomposition of beef tea (Miquel).

Butyric Acid.—Five days' immersion in this acid failed to destroy anthrax spores (Koch).

Arsenious Acid.—A one-per-cent solution destroys the vitality of anthrax spores in ten days, but failed to do so in six days (Koch). In the proportion of 1 : 166 it prevents putrefactive changes in bouillon (Miquel).

Gallic Acid.—Abbott found this acid to destroy the bacteria in broken-down beef tea in the proportion of 2.37 per cent, but it failed to destroy anthrax spores in two hours in the same proportion. Micrococci were killed in two hours by 1 : 142, while 1 : 250 failed.

ALKALIES.

Potassium Hydroxide, KHO.—In the writer's experiments a ten-per-cent solution of caustic potash was fatal to pus cocci, and an eight-per-cent solution failed—two hours' exposure. Exposure for twenty-four hours to a ten-per-cent solution failed to kill the tubercle bacillus (Schill and Fischer). A solution of one per cent kills the anthrax bacillus, the bacillus of rothlauf, and several others (Jäger). The addition of 0.14 per cent restrains the development of the typhoid bacillus, and 0.18 per cent kills this bacillus in four or five hours; the cholera spirillum failed to grow in cultures containing 0.18 per cent and was killed by 0.237 per cent in the same time (Kitasato).

Sodium Hydroxide, NaHO.—The experiments of Jäger and of Kitasato show that soda has about the same germicidal power as caustic potash. Boer obtained the following results with bouillon cultures after two hours' exposure: Anthrax bacillus, 1 : 450 ; diphtheria bacillus, 1 : 300 ; glanders bacillus, 1 : 150 ; typhoid bacillus, 1 : 190 ; cholera spirillum, 1 : 150. In about one-half the amount required to destroy vitality the development of the above-named bacteria was prevented. In the proportion of 1 : 56 it acts as an antiseptic (Miquel).

Ammonia, NH_3.—In Kitasato's experiments the typhoid bacillus was destroyed in five hours by 0.3 per cent of NH_3, and the cholera spirillum by about the same amount. Boer obtained the following results, the time of exposure being two hours : Anthrax bacillus, 1:300 ; diphtheria bacillus, 1 : 250 ; glanders bacillus, 1 : 250 ; typhoid bacillus, 1 : 200 ; cholera spirillum, 1 : 350. The growth of the anthrax bacillus and of the diphtheria bacillus in culture solutions was prevented by 1 : 650.

Calcium Hydroxide, Ca2HO.—According to Kitasato, the typhoid bacillus and the cholera spirillum, in bouillon cultures, are killed in four or five hours by the addition of 0.1 per cent of calcium oxide. Liborius had previously reported still more favorable results, but his bouillon cultures were largely diluted with distilled water. From a practical point of view the experiments of Pfuhl are more valuable. Calcium hydrate was added to the dejections of typhoid patients. When added in the proportion of three per cent sterilization was effected in six hours, and by six per cent in two hours. When milk of lime containing twenty per cent of calcium hydrate was used the results were still more favorable, the typhoid bacillus and cholera spirillum being killed in one hour by the addition of two per cent of the disinfectant. The practical value of lime-wash applied to walls has been determined by Jäger. Silk threads soaked in cultures of various pathogenic bacteria were attached to boards and the lime-wash applied with a camel's-hair brush. Anthrax bacilli (without spores), the glanders bacillus, Staphylococcus pyogenes aureus, and several other pathogenic bacteria were killed by a single application after twenty-four hours, but the tubercle bacillus was not killed by three successive applications. In the writer's experiments (1885) the typhoid bacillus and Staphylococcus pyogenes aureus were killed in two hours by a solution containing 1:40 of calcium oxide, and 1:80 failed. Spores of the anthrax bacillus and of several other spore-forming species were not killed by two hours' exposure to a milk of lime containing twenty per cent of calcium oxide.

Potash Soap has been shown by Jolles (1895) to have considerable germicidal value. In experiments with a soap containing 67.44 per cent of fat acids, 10.4 per cent of combined alkali, and 0.041 per cent of free alkali, the following results were obtained: The typhoid bacillus was destroyed at 18° C. by a one-per-cent solution in twenty-four hours; by a six-per-cent solution in thirty minutes. The Bacillus coli communis required somewhat stronger solutions or longer exposure—eight-per-cent solution required thirty minutes. These experiments show that scrubbing with soap and water is a reliable method of disinfecting surfaces. Solutions of potash—common lye—or of soda also are useful for certain purposes in domestic disinfection, and scientific researches justify the continued use of the cleansing methods which have heretofore been in use by careful housewives.

X.

ACTION OF SALTS.

WHILE some of the metallic salts, and especially those of mercury, silver, and gold, have remarkable germicidal power, others, even in concentrated solutions, do not destroy the vitality of bacteria exposed to their action. For convenience of reference we shall consider the agents in this group in alphabetical order, but first we give Miquel's tables of antiseptic value. This author recognizes the importance of experiments to determine the restraining power of chemical agents for various species of pathogenic bacteria, but says : "As to me, faithful to a plan I adopted at the outset, I will treat the subject in a more general manner by making known simply the minimum weight of the substances capable of preventing the evolution of any bacteria or germs. The method adopted is very simple. To a liquid always comparable to itself it is sufficient at first to add a known weight of the antiseptic and some atmospheric germs or adult bacteria, and to vary the quantity of the antiseptic until the amount is ascertained which will preserve indefinitely the liquid from putrefaction. In order to obtain germs of all kinds in a dry state it suffices to take them, where they are most abundant, in the dust collected in the interior of houses or of hospitals; and to procure a variety of adult bacteria we may take the water of sewers."

SUBSTANCES EMINENTLY ANTISEPTIC.

	Efficient in the proportion of—
Mercuric iodide,	1:40000
Silver iodide,	1:33000
Hydrogen peroxide,	1:20000
Mercuric chloride,	1:14300
Silver nitrate,	1:12500

SUBSTANCES VERY STRONGLY ANTISEPTIC.

Osmic acid,	1:6666
Chromic acid,	1:5000
Chlorine,	1:4000
Iodine,	1:4000
Chloride of gold,	1:4000
Bichloride of platinum,	1:3333
Hydrocyanic acid,	1:2500

Bromine,	1:1666
Cupric chloride,	1:1428
Thymol,	1:1340
Cupric sulphate,	1:1111
Salicylic acid,	1:1000

SUBSTANCES STRONGLY ANTISEPTIC.

Benzoic acid,	1:909
Potassium bichromate,	1:909
Potassium cyanide,	1:909
Aluminum chloride,	1:714
Ammonia,	1:714
Zinc chloride,	1:526
Mineral acids,	1:500 to 1:333
Thymic acid,	1:500
Lead chloride,	1:500
Nitrate of cobalt,	1:476
Sulphate of nickel,	1:400
Nitrate of uranium,	1:356
Carbolic acid,	1:333
Potassium permanganate,	1:285
Lead nitrate,	1:277
Alum,	1:222
Tannin,	1:207

SUBSTANCES MODERATELY ANTISEPTIC.

Bromhydrate of quinine,	1:182
Arsenious acid,	1:166
Boracic acid,	1:143
Sulphate of strychnia,	1:143
Arsenite of soda,	1:111
Hydrate of chloral,	1:107
Salicylate of soda,	1:100
Ferrous sulphate,	1:90
Caustic soda,	1:56

SUBSTANCES FREELY ANTISEPTIC.

Perchloride of manganese,	1:40
Calcium chloride,	1:25
Sodium borate,	1:14
Muriate of morphia,	1:13
Strontium chloride,	1:12
Lithium chloride,	1:11
Barium chloride,	1:10
Alcohol,	1:10

SUBSTANCES VERY FEEBLY ANTISEPTIC.

Ammonium chloride,	1:9
Potassium arsenite,	1:8
Potassium iodide,	1:7
Sodium chloride,	1:6
Glycerin (sp. gr. 1.25),	1:4
Ammonium sulphate,	1:4
Sodium hyposulphite,	1:3

ANTISEPTIC AND GERMICIDAL VALUE OF VARIOUS SALTS, ARRANGED ALPHABETICALLY.

Alum.—Antiseptic in the proportion of 1 : 222 (Miquel).

Aluminum Acetate.—According to De la Croix, this salt is an antiseptic in the proportion of 1 : 6,310. Kuhn found it to be antiseptic in 1 : 5,250.

Aluminum Chloride.—Antiseptic in the proportion of 1 : 714 (Miquel).

Ammonium Carbonate.—When present in the proportion of 1 : 125 it restrains the development of typhoid bacilli, and in five hours' time it kills these bacilli in the proportion of 1 : 100; the cholera spirillum is killed in the same time by 1 : 77 (Kitasato).

Ammonium Chloride.—Antiseptic in the proportion of 1 : 9 (Miquel). A five-per-cent solution does not kill anthrax spores in twenty-five days (Koch).

Ammonium Fluosilicate.—The bacillus of anthrax and of typhoid fever fail to grow in nutrient gelatin containing 1 : 1,000, and a two-per-cent solution kills anthrax spores in one-quarter to three-quarters of an hour (Faktor).

Ammonium Sulphate.—Antiseptic in the proportion of 1 : 4 (Miquel). A five-per-cent solution failed in two days to kill anthrax spores, but was effective in five days (Koch).

Barium Chloride is an antiseptic in the proportion of 1 : 10 (Miquel).

Calcium Chloride is an antiseptic in the proportion of 1 : 25 (Miquel). A saturated solution does not destroy anthrax spores (Koch).

Calcium Hypochlorite.—This is a powerful germicidal agent and has great value as a practical disinfectant. Good chloride of lime contains from twenty-five to thirty per cent of available chlorine as hypochlorite. The experiments made by the Committee on Disinfectants of the American Public Health Association in 1885 showed that a solution containing 0.25 per cent of chlorine as hypochlorite is an effective germicide, even when allowed to act only for one or two minutes. In Bolton's experiments a solution of chloride of lime of 1 : 2,000 (available chlorine 0.015) destroyed the typhoid bacillus and the cholera spirillum in two hours. For the destruction of anthrax spores a one-per-cent solution was required (available chlorine 0.3 per cent). Nissen found that the typhoid bacillus and the cholera spirillum are destroyed with certainty in five minutes by a solution containing 0.12 per cent, anthrax bacilli in one minute by 0.1 per cent, Staphylococcus pyogenes aureus in one minute by 0.2 per cent, anthrax spores in thirty minutes by a five-per-cent solution and in seventy minutes by a one-per-cent solution. Experiments made by the same author upon the sterilization of fæces showed that 0.5 per cent to one per cent could be relied upon to destroy the typhoid bacillus or the cholera spirillum in fæces in ten minutes.

Chloral Hydrate.—Antiseptic in the proportion of 1 : 107 (Miquel). A twenty-per-cent solution destroys pus cocci in two hours (Sternberg).

Cupric Chloride.—Antiseptic in the proportion of 1 · 1,428 (Miquel).

Cupric Sulphate.—Antiseptic in the proportion of 1 : 111 (Miquel). Kills the cholera spirillum in the proportion of 1 : 3,000 in ten minutes (Nicati and Rietsch). Destroys the cholera spirillum in bouillon cultures in less than half an hour in 1 : 600, and in four hours in 1 : 1,000 ; cultures in blood serum require 1 : 200 (Van Ermengem). A solution of 1 : 20 kills the typhoid bacillus in ten minutes (Leitz). This salt failed, in the writer's experiments, to kill the spores of Bacillus anthracis and Bacillus subtilis in two hours' time in a twenty-per-cent solution. In Koch's experiments a five-per-cent solution failed to kill anthrax spores in ten days. Kills pus micrococci in two hours in the proportion of 1 : 200 (Sternberg). In Bolton's experiments made for the Committee on Disinfectants of the American Public Health Association the following results were obtained: Recent cultures in bouillon, time of exposure two hours : Bacillus of typhoid fever, 1 : 200; cholera spirillum, 1 : 500; Bacillus pyocyanus, 1 : 200; Brieger's bacillus, 1 : 200; Emmerich's bacillus, 1 : 200; Staphylococcus pyogenes aureus, 1 : 100 ; Staphylococcus pyogenes citreus, 1 : 100; Staphylococcus pyogenes albus, 1 : 200; Streptococcus pyogenes, 1 : 500. When ten per cent of dried egg albumin was added to a recent culture in bouillon of the typhoid bacillus the amount required to insure sterilization was 1 : 10.

In the report of the Committee on Disinfectants of the American Public Health Association this agent is recommended in "a solution of two to five per cent for the destruction of infectious material *not containing spores.*" The experimental data above given show that this is a liberal allowance for material which does not contain an excessive amount of albumin. In the experiments of Leitz the typhoid bacillus in cultures was destroyed in ten minutes by a five-per-cent solution.

Ferric Chloride.—A five-per-cent solution failed in two days to destroy anthrax spores, but was effective in five days (Koch).

Ferrous Sulphate.—In the writer's experiments (1883) a solution of twenty per cent failed to destroy micrococci and putrefactive bacteria. In a more recent experiment ten per cent failed to kill pus cocci, but was fatal to Micrococcus tetragenus—two hours' exposure. Koch found that a five-per-cent solution failed to destroy anthrax spores in six days. Exposure to a twenty-per-cent solution for forty-eight hours does not destroy the virus of symptomatic anthrax (Arloing, Cornevin, and Thomas). In the experiments of Jäger immersion in a solution of 1 : 3 destroyed the infective virulence of certain pathogenic bacteria (fowl cholera, rothlauf, glanders), as tested by injection into mice, but failed to kill anthrax spores and tubercle bacilli. The antiseptic power of ferrous sulphate is placed by Miquel at 1 : 90. In the writer's experiments 1 : 200 prevented the development of micrococci and of putrefactive bacteria in bouillon placed in the incubating oven for forty-eight hours. Leitz found that a five-per-cent solution required three days' exposure for the destruction of the typhoid bacillus.

Gold Chloride.—Antiseptic in the proportion of 1 : 4,000 (Miquel). Boer has made extended experiments with the chloride of gold and sodium. We give his results below. In his disinfection experiments a bouillon culture which had been in the incubating oven for twenty-four hours was used, and the time of exposure was two hours.

	Restrains development.	Destroys vitality.
Anthrax bacillus	1 : 40000	1 : 8000
Diphtheria bacillus	1 : 40000	1 : 1000
Glanders bacillus	1 : 15000	1 : 400
Typhoid bacillus	1 : 20000	1 : 500
Cholera spirillum	1 : 25000	1 : 1000

Lead Chloride.—Antiseptic in the proportion of 1 : 500 (Miquel).

Lead Nitrate.—Antiseptic in the proportion of 1 : 277 (Miquel).

Lithium Chloride.—Antiseptic in the proportion of 1 : 11 (Miquel).

Manganese Protochloride.—Antiseptic in the proportion of 1 : 40 (Miquel).

Mercuric Chloride.—Koch's experiments (1881) gave the following results: A solution of 1 : 1,000 destroys anthrax spores in a few minutes, and 1 : 10,000 is effective after a more prolonged exposure. The writer (1884) obtained similar results—1 : 10,000 destroyed the spores of Bacillus anthracis and of Bacillus subtilis in two hours. More recent experiments indicate that failure to grow in culture solutions cannot be accepted as evidence of the destruction of vitality in the case of spores exposed to the action of this agent, unless due precautions are taken to exclude the restraining influence of the small amount of mercuric chloride which remains attached to the spores. Koch had ascertained that the development of spores is restrained by the presence of 1 : 300,000 in a culture medium, and Geppert has recently shown that even so small an amount as 1 : 2,000,000 will prevent the development of spores the vitality of which has been reduced by the action of a strong solution (1 : 1,000). When this restraining action is entirely neutralized by washing the spores in a solution containing ammonium sulphide it requires, according to Geppert, a solution of 1:1,000 acting for one hour to completely destroy the vitality of anthrax spores. Fränkel found that a solution of 1 : 1,000 was effective in half an hour. The typhoid bacillus, the bacillus of mouse septicæmia, and the cholera spirillum, in bouillon cultures and in cultures in flesh-peptone-gelatin, are destroyed in two hours by 1 : 10,000 ; but in a bouillon culture to which ten per cent of dried egg albumin was added a one-per-cent solution was required to destroy the typhoid bacillus in the same time (Bolton). According to Van Ermengem, cultures of the cholera spirillum in bouillon are sterilized in half an hour by 1 : 60,000, but cultures in blood serum require 1 : 800 to 1 : 1,000. In experiments upon tuberculous sputum Schill and Fischer found that exposure of fresh sputum to an equal amount of a 1 : 2,000 solution for twenty-four hours failed to disinfect it, as shown by inoculation experiments in guinea-pigs. The antiseptic power of mercuric chloride is given by Miquel as 1 : 14,300. In the writer's experiments 1 : 33,000 was found to prevent the development of putrefactive bacteria in bouillon, but a minute bacillus contained in broken-down beef infusion multiplied, after several days, in 1 : 20,000. The pus cocci were restrained in their development by 1 : 30,000.

In Behring's experiments the anthrax bacillus and cholera spirillum were killed in one hour by 1 : 100,000 when the temperature was 36° C., but at a temperature of 3° C. the proportion required was 1 : 25,000. The same author states that at 22° C. Staphylococcus aureus in bouillon is not always killed in twenty-five minutes by 1 : 1,000.

Abbott (1891) has shown that a 1 : 1,000 solution does not always destroy Staphylococcus pyogenes aureus in five minutes. He says: "Frequently all the organisms would be destroyed after five minutes' exposure, but almost as often a certain few would resist for that length of time, and even longer, going in some cases to ten, twenty, and even thirty minutes."

According to Yersin, a solution of 1 : 1,000 kills the tubercle bacillus in one minute.

We might add considerably to the experimental data given, but the results already recorded are sufficient to show the value of this agent as an antiseptic and germicide, and justify its use for general purposes of disinfection in the proportion of 1 : 500 or 1 : 1,000 for material containing spores, and in the proportion of 1 : 2,000 to

1 : 5,000 for pathogenic bacteria in the absence of spores; due regard being had to the fact that the presence of albumin very materially reduces its germicidal potency, and that it may be decomposed and neutralized by alkalies and their carbonates, by hydrosulphuric acid, and by many other substances.

The albuminate of mercury, as has been shown by Lister, is soluble in an excess of albumin, and, according to Behring, is just as effective as an aqueous solution containing the same amount of sublimate when dissolved in an albuminous liquid like blood serum (?).

In practice the addition of a mineral acid to sublimate solutions, or of sodium, potassium, or ammonium chloride, is to be recommended, to prevent the precipitation of the mercuric chloride by albumin in fluids containing it. Behring recommends the addition of five parts of sodium or potassium chloride to one of the sublimate. Such a solution is more stable than a simple solution of sublimate, and no precipitate is formed by the addition of alkalies or by albumin.

The same result is obtained, according to La Place, by the addition of five parts of hydrochloric or tartaric acid to one part of sublimate in aqueous solution.

Mercuric Cyanide, $Hg(CN)_2$, and the *Oxycyanide* of mercury have been tested, with the following results : Staphylococcus aureus is destroyed in five minutes by 1 : 100, in one hour by 1 : 1,000, in two hours by 1 : 1,500 (Chibret). The development of Bacillus anthracis in culture solutions is prevented by the presence of cyanide of mercury in the proportion of 1 : 25,000, and by the oxycyanide by 1 : 16,000 (Behring).

Boer obtained the following results with the oxycyanide—cultures in bouillon, twenty-four hours in incubating oven, time of exposure two hours :

	Restrained development.	Destroyed vitality.
Anthrax bacillus	1 : 80000	1 : 40000
Diphtheria bacillus	1 : 80000	1 : 40000
Glanders bacillus	1 : 60000	1 : 30000
Typhoid bacillus	1 : 60000	1 : 30000
Cholera spirillum	1 : 90000	1 : 60000

Mercuric Iodide.—The antiseptic value of this salt is placed by Miquel at 1 : 40,000, which is more than double that given by the same author to the bichloride. In the writer's experiments upon the antiseptic value of salts and oxides of mercury the following results were obtained :

	Active.	Failed.
Biniodide of mercury	1 : 20000	1 : 40000
Bichloride	1 : 15000	1 : 20000
Protiodide	1 : 10000	1 : 20000
Yellow oxide	1 : 1000	1 : 2000
Black oxide	1 : 500	1 : 1000

Morphia Hydrochlorate.—Antiseptic in the proportion of 1 : 13 (Miquel).

Nickel Sulphate.—Antiseptic in the proportion of 1 : 400 (Miquel).

Platinum Bichloride.—Antiseptic in the proportion of 1 : 3,333 (Miquel).

Potassium Acetate.—A saturated solution of this salt failed to kill anthrax spores in ten days (Koch).

Potassium Arsenite.—In the writer's experiments Fowler's solution failed to kill micrococci in two hours in the proportion of four per cent. Miquel places the antiseptic value of potassium arsenite at 1 : 8.

Potassium Bichromate.—A five-per-cent solution failed in two days to destroy anthrax spores (Koch). Efficient as an antiseptic in the proportion of 1 : 909 (Miquel).

Potassium Bromide.—The bacillus of typhoid fever and the cholera spirillum fail to grow in culture solutions containing 9 to 10.6 per cent, and are killed in four or five hours by ten to twelve per cent (Kitasato).

Potassium Carbonate.—The development of the typhoid bacillus and of the cholera spirillum is prevented by 0.74 to 0.81 per cent, and these bacteria are killed in five hours by 1 per cent (Kitasato).

Potassium Chlorate.—In the writer's experiments a four-per-cent solution failed in two hours to kill Micrococcus Pasteuri. A five-per-cent solution failed in six days to destroy anthrax spores (Koch).

Potassium Chromate.—A five-per-cent solution failed to kill anthrax spores in five days (Koch).

Potassium Cyanide.—Antiseptic in the proportion of 1 : 909 (Miquel).

Potassium Iodide.—A solution of five per cent does not destroy anthrax spores in eighty days (Koch). Putrefactive bacteria in broken-down beef infusion are not destroyed by two hours' exposure in a twenty-per-cent solution (Sternberg). The typhoid bacillus and the cholera spirillum do not grow in culture solutions containing eight per cent, and are destroyed by five hours' exposure to 9.23 per cent (Kitasato). Antiseptic in the proportion of 1 : 7 (Miquel).

Potassium Permanganate.—In the writer's experiments (1881) a two-per-cent solution was required to destroy Micrococcus Pasteuri in the blood of a rabbit. In later experiments pus cocci in bouillon were killed by 1 : 833—time of exposure two hours. One per cent was found by Koch not to destroy anthrax spores in two days, but five per cent was effective in one day. The glanders bacillus is destroyed in two minutes by a one-per-cent solution (Löffler). The experiments of Jäger show that a one-per-cent solution is not reliable for the destruction of anthrax bacilli and other pathogenic bacteria tested, but a five-per-cent solution was effective. The tubercle bacillus was not, however, killed by exposure in a five-per-cent solution. According to Miquel, permanganate of potash is an antiseptic in the proportion of 1 : 285.

Quinine Hydrobromate.—Antiseptic in the proportion of 1 : 182 (Miquel).

Quinine Hydrochlorate.—Antiseptic in the proportion of 1 : 900 (Ceri). Quinine dissolved with hydrochloric acid destroys anthrax spores in ten days in one-per-cent solution (Koch).

Quinine Sulphate.—The writer found that in the proportion of 1 : 800 quinine prevents the development of various micrococci and bacilli. A ten-per-cent solution does not destroy the bacilli of symptomatic anthrax (Arloing, Cornevin, and Thomas).

Silver Nitrate.—Miquel places nitrate of silver next to mercuric chloride as an antiseptic, effective in the proportion of 1 : 12,500. Behring also places it next to bichloride as an antiseptic and germicide, and says that it is even superior to this salt in albuminous fluids. He reports that it prevents the development of anthrax spores when present in a culture liquid in the proportion of 1: 80,000, and in the proportion of 1 : 10,000 destroys these spores in forty-eight hours. We give below the result of recent experiments by Boer, in which the time of exposure was two hours:

	Restrains development.	Destroys vitality.
Anthrax bacillus	1 : 60000	1 : 20000
Diphtheria bacillus	1 : 60000	1 : 2500
Glanders bacillus	1 : 75000	1 : 4000
Typhoid bacillus	1 : 50000	1 : 4000
Cholera spirillum	1 : 50000	1 : 4000

Silver Chloride.—A solution of chloride of silver in hyposulphite of soda is much less effective as an antiseptic than nitrate of silver.

Behring found that to prevent the development of anthrax spores a solution of 1 : 8,000 was required.

Sodium Borate.—In the writer's experiments a saturated solution of borax was found to be without germicidal power. A twenty-per-cent solution does not destroy the virus of symptomatic anthrax (Arloing, Cornevin, and Thomas). A five-per-cent solution failed to destroy anthrax spores in fifteen days (Koch). Antiseptic in the proportion of 1 : 14 (Miquel).

Sodium Carbonate.—A solution of 2.2 per cent restrains the growth of the typhoid bacillus, and of 2.47 per cent of the cholera spirillum. The first-named bacillus is killed by four or five hours' exposure in a 2.47-per-cent solution, and the cholera spirillum by 3.45 per cent (Kitasato).

Sodium Chloride.—A saturated solution failed in forty-eight hours to destroy the virus of symptomatic anthrax (Arloing, Cornevin, and Thomas). A saturated solution failed in forty days to destroy anthrax spores (Koch). A saturated solution failed in twenty hours to destroy the tubercle bacillus in fresh sputum (Schill and Fischer). In the writer's experiments a five-per-cent solution failed to kill Micrococcus Pasteuri in blood. Antiseptic in the proportion of 1 : 6 (Miquel). According to Forster, the bacillus of typhoid fever, the bacillus of rouget, and the streptococcus of pus are not killed by several weeks' exposure in strong solutions of sodium chloride, but the cholera spirillum is destroyed in a few hours. Cultures of the tubercle bacillus are not sterilized in two months by a saturated solution; and tuberculous organs from an ox, preserved in a solution of salt, did not lose their power of infecting susceptible animals inoculated with material from the diseased tissue. The flesh of swine which died of rothlauf was found by Petri to still contain the bacillus in a living condition after having been preserved in brine for a month.

Sodium Hyposulphite.—In the writer's experiments a saturated solution failed in two hours to kill micrococci and bacilli. Exposure for forty-eight hours to a fifty-per-cent solution does not destroy the virus of symptomatic anthrax (Arloing, Cornevin, and Thomas). Antiseptic in the proportion of 1 : 3 (Miquel).

Sodium Sulphite.—The results with a saturated solution of this salt were, in the writer's experiments, entirely negative.

Tin Chloride.—A one-per-cent solution acting for two hours destroyed the bacteria in putrefying bouillon, while 0.8 per cent failed (Abbott).

Zinc Chloride.—In the writer's experiments 1 : 200 destroyed Micrococcus Pasteuri in two hours, but a two-per-cent solution was required to kill pus cocci in the same time; spores of Bacillus anthracis were not destroyed by two hours' exposure in a ten-per-cent solution, but a solution of five per cent killed the spores of Bacillus subtilis in the same time. Koch found that anthrax spores germinated after being immersed in a five-per-cent solution for thirty days. The development of Bacillus prodigiosus is only slightly retarded by exposure for sixteen hours in a one-per-cent solution. Antiseptic in the proportion of 1 : 526 (Miquel).

Zinc Sulphate.—In the writer's first experiments a twenty-per-cent solution failed to destroy in two hours micrococci obtained from the pus of an acute abscess. In later experiments a micrococcus from the same source resisted two hours' exposure to a ten-per-cent solution, but Micrococcus tetragenus was destroyed by this amount. Broken-down beef infusion mixed with an equal quantity of a forty-per-cent solution was not sterilized after two hours' contact. In Koch's experiments anthrax spores were found to germinate after having been immersed for ten days in a five-per-cent solution.

XI.

ACTION OF COAL-TAR PRODUCTS, ESSENTIAL OILS, ETC.

In the present section we shall consider the action upon bacteria of a variety of organic products, and for convenience will arrange them alphabetically.

Acetone.—Anthrax spores grow freely after two days' exposure to the action of this agent; at the end of five days their development is feeble (Koch).

Alcohol.—In the writer's experiments ninety-five-per-cent alcohol did not destroy the bacteria (spores) in broken-down beef tea in forty-eight hours. Micrococcus Pasteuri was destroyed by two hours' exposure in a twenty-four-per-cent solution ; pus cocci required a forty-per-cent solution. Koch found that absolute alcohol had no effect upon anthrax spores exposed to its action for one hundred and ten days. Schill and Fischer found that when tuberculous sputum was mixed with an equal amount of absolute alcohol its infecting power was not destroyed in twenty-four hours, but that in the proportion of five parts to one of sputum it was effective in destroying the tubercle bacillus, as proved by inoculation experiments. Yersin found that in pure cultures the tubercle bacillus is killed by five minutes' exposure to the action of absolute alcohol.

Aniline Dyes.—Recent researches have shown that some of the aniline colors possess very decided germicidal power. Stilling found that solutions of methyl violet containing 1 : 30,000 exercise a restraining influence upon the development of putrefactive bacteria and pus cocci, and that these microörganisms are destroyed by solutions containing 1 : 2,000 to 1 : 1,000. Methyl violet has been placed in the market by Merck under the name of pyoktanin. Jänicke reports the following results with pyoktanin : Staphylococcus pyogenes aureus was restrained in its development by solutions containing 1 : 2,000,000, Bacillus anthracis by 1 : 1,000,000, Staphylococcus pyogenes by 1 : 333,300, Spirillum choleræ Asiaticæ by 1 : 62,500, Bacillus typhi abdominalis by 1 : 5,000. In blood serum stronger solutions were required (1 : 500,000 for Staphylococcus pyogenes aureus). Staphylococcus pyogenes aureus, Streptococcus pyogenes, and Bacillus anthracis were killed in thirty seconds by 1 : 1,000, the typhoid bacillus by the same amount in thirty minutes. Boer found malachite green to be still more effective than methyl violet. In his experiments upon bouillon cultures twenty-four hours old, with two hours' exposure to the action of the disinfectant, he obtained the following results :

MALACHITE GREEN.

	Restrains development.	Destroys vitality.
Anthrax bacillus	1 : 120000	1 : 40000
Diphtheria bacillus	1 : 40000	1 : 8000
Glanders bacillus	1 : 5000	1 : 300
Typhoid bacillus	1 : 5000	1 : 300
Cholera spirillum	1 : 100000	1 : 5000

METHYL VIOLET (PYOKTANIN).

	Restrains development.	Destroys vitality.
Anthrax bacillus	1 : 70000	1 : 5000
Diphtheria bacillus	1 : 10000	1 : 2000
Glanders bacillus	1 : 2500	1 : 150
Typhoid bacillus	1 : 2500	1 : 150
Cholera spirillum	1 : 30000	1 : 1000

Aniline Oil.—According to Riedlin, the addition of 1 : 5 of aniline water prevents the development of all bacteria in nutrient gelatin.

Aromatic Products of Decomposition.—Klein has tested the germicidal power of phenylpropionic and phenylacetic acids. He finds that anthrax spores resist both of these acids, in the proportion of 1 : 400, for two days, but in the absence of spores anthrax bacilli are quickly killed by a solution of this strength. Certain non-pathogenic micrococci were not killed by exposure for twenty-five minutes to 1 : 200. The caseous matter of pulmonary tuberculosis infected guinea-pigs after exposure for ninety-six hours to 1 : 200.

Aseptol.—A ten-per-cent aqueous solution kills anthrax spores in ten minutes, and a three- to five-per-cent solution is a reliable disinfectant in the absence of spores (Hueppe).

Benzene, C_6H_6.—Exposure in benzol for twenty days failed to destroy the vitality of anthrax spores (Koch).

Camphor.—Alcohol saturated with camphor has no effect upon the virus of symptomatic anthrax (Arloing, Cornevin, and Thomas)

The experiments of Cadéac and Meunier show that camphor (oil of, or tincture?) has but little germicidal power. The typhoid bacillus and cholera spirillum were only destroyed after eight to ten days' exposure to the action of camphor ("essence").

Carbolic Acid.—Tested upon anthrax spores, Koch found a one-per-cent solution to be without effect after fifteen days' exposure; a two-per-cent solution retarded development but did not completely destroy vitality in seven days; a three-per-cent solution was effective in two days. In the absence of spores Koch found that a one-per-cent solution quickly destroys the vitality of anthrax bacilli. He recommends a five-per-cent solution for the destruction of the "comma bacillus" in the discharges of cholera patients, and a two-per-cent solution for the disinfection of surfaces soiled with such discharges. In the writer's experiments 1 : 200 destroyed Micrococcus Pasteuri in two hours; and pus cocci were destroyed by 1 : 125, while 1 : 200 failed. Davaine showed by inoculation experiments that anthrax bacilli in fresh blood are destroyed by being exposed to the action of a one-per-cent solution for one hour. A two-per-cent solution destroys the dried virus of symptomatic anthrax in forty-eight hours (Arloing, Cornevin, and Thomas). Solutions in oil or in alcohol have been shown by Koch to be less effective than aqueous solutions. Thus a five-per-cent solution in oil failed to destroy anthrax spores in one hundred and ten days, and the same solution failed to kill the bacilli, in the absence of spores, in less than six days. A five-per-cent solution in alcohol did not destroy anthrax spores in seventy days. Schill and Fischer found that a three-per-cent solution destroyed the infecting power of tuberculous sputum, as shown by inoculation into guinea-pigs, in twenty-four hours, while solutions of one and two per cent failed. Bolton's experiments gave the following results, the test organisms being in fresh bouillon cultures and the time of exposure two hours: The cholera spirillum, the bacillus of typhoid fever, the bacillus of schweinerothlauf, Brieger's bacillus, the bacillus of green pus, and the pus cocci (Staphylococcus pyogenes aureus, albus, and citreus, and Streptococcus pyogenes) were all killed by a solution of one per cent, while in a majority of the experiments a one-half-per-cent (1 : 200) solution failed. Cultures of the typhoid bacillus in flesh-peptone-gelatin gave the same result (1 : 100 with two hours' exposure), and the addition of ten per cent of dried egg albumin to bouillon cultures did not influence the result.

The experiments of La Place show that the addition of hydrochloric acid to a disinfecting solution containing carbolic acid greatly increases its germicidal power for spores. Thus it is stated that "two per cent of crude carbolic acid with one per cent of pure hydrochloric acid destroyed anthrax spores in seven days, while two per cent of carbolic acid or one per cent of hydrochloric acid alone did not destroy these spores in thirty days. A four-per-cent solution of crude carbolic acid with two per cent of hydrochloric acid destroyed spores in less than an hour; four per cent of carbolic acid alone did not destroy them in twelve days. Van Ermengem reports that in his experiments the cholera spirillum in chicken bouillon was killed in less than half an hour by 1 : 600, and that in blood serum 1 : 400 was effective. Nicati and Rietsch fix the germicidal power for the cholera spirillum as 1 : 200, the time of exposure being ten minutes; Ramon and Cajal, 1 : 50. Boer gives the following results, the time of exposure being two hours, cultures in bouillon twenty-four hours old :

	Restrains development.	Destroys vitality.
Anthrax bacillus	1 : 750	1 : 300
Diphtheria bacillus	1 : 500	1 : 300
Glanders bacillus	1 : 500	1 : 300
Typhoid bacillus	1 : 400	1 : 200
Cholera spirillum	1 : 600	1 : 400

Leitz reports the following results : The dejections of patients suffering from typhoid fever, mixed in equal quantity with the disinfecting solution, were sterilized by a five-per-cent solution of carbolic acid in three days. Pure cultures of the typhoid bacillus were sterilized in fifteen minutes by a five-per-cent solution.

In the experiments of Nocht upon anthrax spores it was found that while at the room temperature these spores were not destroyed by several days' exposure in a five-per-cent solution, they were destroyed in three hours by the same solution at a temperature of 37.5°.

Carbolic acid prevents putrefactive changes in bouillon when present in the proportion of 1 : 333 (Miquel). The tubercle bacillus is killed in thirty seconds by a five-per-cent solution, and in one minute by a one-per-cent solution (Yersin).

Coffee Infusion.—Experiments have been made by Heim and by Lüderitz on the antiseptic power of an infusion of coffee. The first-named author found that anthrax bacilli no longer developed after three hours' exposure in a ten-per-cent solution, but spores were not killed at the end of a week. Streptococci in a bouillon culture required twenty-four hours' exposure, and the staphylococci of pus were not destroyed in this time. Lüderitz found that a three-per-cent infusion restrained the growth in nutrient gelatin of the typhoid bacillus, and a five-per-cent infusion killed the bacillus in two days; the cholera spirillum failed to grow in presence of one per cent, and a solution of this strength killed it in seven hours; Staphylococcus pyogenes aureus was prevented from developing by two per cent, and was killed in six days by a five-per-cent solution ; Streptococcus pyogenes was prevented from growing by one per cent, and killed by a ten-per-cent solution in one day ; Proteus vulgaris did not grow in presence of 2.5 per cent, and was killed in two days by ten per cent. The question as to what constituent of the infusion of roasted coffee was the active germicidal agent was not determined, but the authors referred to agree that it was not caffeine.

Creolin.—This is a coal-tar product which resembles crude carbolic acid in appearance, but smells rather like tar than like phenol. It makes a milky emulsion with water, which has been proved by numerous experiments to possess very decided germicidal power, being superior to carbolic acid. The first careful test of the germicidal power of this agent was made by Esmarch, who found that a solution of 1 : 200 killed the cholera spirillum in a minute, the typhoid bacillus at the end of several days. Anthrax spores were not destroyed in twenty days by a five-per-cent solution, but this solution killed the tubercle bacillus attached to silk threads which were immersed in it for a short time, and also disinfected tuberculous sputum. Behring has shown that in albuminous liquids creolin is less effective than carbolic acid. In blood serum 1 : 175 was required to restrain the development of staphylococci, and 1 : 100 to destroy the same in ten minutes. Van Ermengem, as a result of numerous experiments, arrived at the conclusion that creolin is a cheap and useful disinfecting agent, in a five-per-cent solution, for various pathogenic organisms. Kaupe reports that in his experiments a ten-per-cent solution killed anthrax spores in twenty-four hours. According to Boer, a solution of 1 : 5,000 destroys anthrax bacilli in bouillon cultures in two hours, 1 : 2,000 diphtheria bacilli, 1 : 300 the glanders bacillus, 1 : 250 the typhoid bacillus, and 1 : 3,000 the cholera spirillum.

Creosote.—This agent was found by the writer to be fatal to micrococci in the proportion of 1 : 200. In the proportion of one per cent it failed, after twenty hours' exposure, to destroy tubercle bacilli in sputum (Schill and Fischer). A saturated aqueous solution does not destroy the tubercle bacillus in cultures in twelve hours (Yersin). Guttman, in extended experiments upon various pathogenic organisms, found that development was prevented by 1 : 3,000 to 1 : 4,000. A solution containing 1 : 300 killed Bacillus pyocyanus and Bacillus anthracis in one minute, Bacillus prodigiosus in two minutes, and the Finkler-Prior spirillum in one minute in the proportion of 1 : 600.

Cresol.—This is a dark, reddish-brown, transparent fluid, somewhat thinner than creolin, and, like it, having an odor of tar. It forms an emulsion with water, which is not so stable as that formed by creolin. Of the three cresols, ortho-, meta-, and paracresol, the second was found by Fränkel to be most active. This author states that the addition of sulphuric acid adds greatly to its germicidal power. A four-per-cent solution, containing equal parts of cresol and H_2SO_4, killed anthrax spores in less than twenty-four hours. In Behring's experiments a solution containing ten per cent of each killed anthrax spores in eighty minutes, and five per cent of each in one hundred minutes, while an eighteen-per-cent solution of sulphuric acid alone did not kill them in twenty four hours. In the experiments of Jäger a two-per-cent solution destroyed the tubercle bacillus in cultures and in sputum. As a result of his experiments Behring concludes that cresol has no advantage over carbolic acid as a germicide for the destruction of spores. Tested upon Staphylococcus aureus, Streptococcus erysipelatos, and Bacillus pyocyanus, Fränkel found that a solution of 0.3 per cent destroyed these microörganisms in five minutes, while a two-per-cent solution of carbolic acid required fifteen minutes' contact to accomplish the same result.

Trikresol (Schering) has been tested, with favorable results, by several bacteriologists. According to Hammerl it is about twice as active a germicide as carbolic acid.

Diaphtherin (oxychinaseptol) has considerable antiseptic power, as shown by the experiments of Rohrer and others. Two to four drops of a one-per-cent solution was found to prevent the development of test organisms (Staphylococcus pyogenes aureus and Bacillus anthracis) in twelve cubic centimetres of bouillon. Stahle (1893) also finds that as an antiseptic it is far superior to carbolic acid or lysol, and that it has the advantage of being non-toxic. Tested upon anthrax spores it was found to be comparatively inactive as a germicide. A fifteen-per-cent solution destroyed anthrax spores in three days.

Disinfektol.—This is a coal-tar product similar to creolin which has been recommended in Germany for disinfecting purposes. It is an oily, dark-brown fluid having a specific gravity of 1.086. It forms an emulsion with water, which has a slightly alkaline reaction. It has been tested upon typhoid stools by Uffelmann and by Beselin. The last-named author gives the following summary of the results obtained : An emulsion of five per cent of disinfektol equals in value, for the disinfection of the liquid discharges of typhoid patients, 12.5 per cent of creolin, thirty-three per cent of hydrochloric acid, five per cent of carbolic acid, 1 : 500 of mercuric chloride.

Ether.—Anthrax spores may germinate after being immersed in sulphuric ether for eight days (Koch). The tubercle bacillus is destroyed by ten minutes' exposure to the action of ether (Yersin).

Essential Oils.—Chamberlain has made an extended series of experiments to determine the antiseptic power of the vapor of volatile oils. A large number of essential oils tested were found to prevent the development of the anthrax bacillus, while a few did not. At the end of six days the tubes were opened and the oil absorbed by the culture liquid allowed to evaporate. Cultures were now obtained from all except the following, which, it was inferred, had destroyed the vitality of the spores: Angelica, cinnamon of China, cinnamon of Ceylon, geranium of France, geranium of Algeria, origanum.

Cadéac and Meunier have also made extended experiments upon the typhoid bacillus and the bacillus of glanders, for the purpose of determining the germicidal power of agents of this class. Their method consisted in the introduction of a sterilized platinum needle into a pure culture of the test organism, in immersing it in the essential oil for a certain time, and then making with it a puncture in a suitable solid culture medium. Their results are given below for the typhoid bacillus.

Essences which kill the bacillus after a contact of less than twenty-four hours:

	At the end of—
Cinnamon of Ceylon,	12 minutes.
Cloves,	25 "
Eugenol,	30 "
Thyme,	35 "
Wild thyme,	35 "
Verbena of India,	45 "
Geranium of France,	50 "
Origanum,	75 "
Patchouly,	80 "
Zedoary,	2 hours.
Absinthe,	4 "
Sandalwood,	12 "

The following were effective in from twenty-four to forty-eight hours: Cumin, caraway, juniper, matico, galbanum, valerian, citron, angelica, celery, savin, copaiba, pepper, turpentine, opopanax, rose, chamomile; the following required from two to four days: Illicium, sassafras, tuberose, coriander; the following from four to eight days: Calamus, sage, fennel, mace, cascarilla, orange of Portugal; the following in eight to ten days: Mint, nutmeg, rosemary, carrot, mustard, anise, onion, marjoram, bitter almonds, cherry laurel, myrtle, lavender, eucalyptus, cedar, cajuput, wintergreen, camphor.

Riedlin reports as the result of his experiments that the essential oils which have the greatest antiseptic value are oil of lavender, eucalyptus, rosemary, and cloves.

Eucalyptol.—Chabaunes and Perret found that a five-per-cent solution of eucalyptol is without effect upon tubercle bacilli in sputum. According to Behring, eucalyptol is about four times less active as a disinfectant than carbolic acid.

Euphorin (Phenylurethan) has been tested by Colasanti (1894), who finds that it has rather feeble germicidal activity.

Formaldehyde (formol, formalin) has very decided germicidal power. According to Pottevin (1894) in the absence of spores a solution of 1:1,000 kills bacteria, in comparatively small numbers, in from fifteen minutes to several hours. For the destruction of spores a much stronger solution is required—a fifteen-per-cent solution at 15° C. killed anthrax spores in one and one-half hours, and spores of Bacillus subtilis in twenty hours. At higher temperatures the germicidal action is more energetic, and microörganisms exposed to the vapor of formol are very quickly destroyed. Vanderlinden and de Buck (1895) find that solutions of formalin are decidedly inferior to corresponding solutions of carbolic acid, creolin, or solvéol, and are too irritating to be used in surgical practice. They report that a solution of five per cent failed to destroy their test organisms—Bacillus coli communis, Bacillus typhi abdominalis, Staphylococcus pyogenes aureus. Experiments made by Reed, at the Army Medical Museum in Washington, show that the diphtheria bacillus and other test organisms are quickly killed by formalin vapor.

Glycerin has no action upon the virus of symptomatic anthrax (Arloing, Cornevin, and Thomas), and is inert as regards the spores of anthrax (Koch). Glycerin prevents putrefactive decomposition in bouillon when present in the proportion of 1:4 (Miquel). Roux has shown that the addition of five per cent of glycerin to a culture medium is favorable to the growth of the tubercle bacillus; it is also appropriated as pabulum by various other species.

Guaiacol.—Kuprianow, as a result of extended experiments with this agent (1894), reports that it ranks below cresol and carbolic acid as a germicide. In the proportion of 1:500 it restrains the development of the cholera spirillum, and the author named suggests its internal administration in this disease on account of its non-toxic and non-irritant properties.

Hydroxylamin.—Heinisch found that the development of the anthrax bacillus is prevented by 1:77 of hydroxylamin hydrochlorate, and of the diphtheria bacillus by 1:75. In these experiments a solution of soda was added to release the hydroxylamin. Marpmann found that 1:100 preserved milk without change for four to six weeks, and that alkaline fermentation of urine was prevented by 1:1,000.

Ichthyol.—Latteux (1892) reports that the various pathogenic bacteria used by him as test organisms were killed by a five-per-cent solution (time ?) with exception of Streptococcus pyogenes, which required a six to seven-per-cent solution. The more recent experiments of Abel (1893) gave less favorable result, but the agent was shown to have considerable antiseptic value—1:2,000 restrained the development of streptococci; 1:500 of the diphtheria bacillus; 1:20 of Staphylococcus pyogenes aureus; 1:33 the bacillus of typhoid fever. Streptococci and diphtheria bacilli were destroyed in twenty-four hours by a solution of 1:200; Staphylococcus aureus, subjected to the action of pure ichthyol, was destroyed in five hours—in a five-per-cent solution it survived for four days. Cultures of the typhoid bacillus mixed with a fifty-per-cent solution were not completely sterilized in thirty hours; a small number of bacilli in bouillon were, however, destroyed by a three-per-cent solution in forty-eight hours. Anthrax spores on silk threads were not destroyed by a fifty-per-cent solution at the end of one hundred and forty days.

Indol.—When added in excess to water this agent failed to destroy anthrax spores in eighty days (Koch).

Izal is a coal-tar product which has recently been introduced as a disinfectant. Klein (1892) reports that in the strength of ten per cent it kills anthrax spores in fifteen minutes. In the absence of spores various pathogenic bacteria were killed in five minutes by a solution containing 1:200.

Lanolin.—According to Gottstein, various microörganisms tested by him failed to grow in cultures after having been in contact with pure lanolin for five to seven days.

Loretin.—Korff (1895) claims for this agent that a two-per-cent solution is superior to corresponding solutions of lysol, metakresol, or phenol, and that it has the advantage of being non-toxic, odorless, and non-irritating.

Lysol.—Weiss (1895) has tested this product and reports that a solution of three-fourths per cent destroyed his test organisms (pus cocci, typhoid bacillus, Bacillus coli communis, etc.) in five minutes. Anthrax spores were destroyed by the same solution in one hour.

Naphthol.—In the proportion of 1:10,000 naphthol prevents the development of the glanders bacillus, the anthrax bacillus, the typhoid bacillus, the micrococcus of fowl cholera, of Staphylococcus aureus and albus, and of several other microörganisms tested by Maximovitch. The same author states that although insoluble in cold water, water at 70° C. dissolves 0.44 in one thousand parts. When urine is shaken up with naphthol in powder it does not undergo fermentation.

In the experiments of Foote hydronaphthol was found to show some germicidal power in the proportion of 1:2,300, but the conclusion is reached that a saturated aqueous solution (1:1,150) does not equal a one-per-cent solution of carbolic acid or of creolin.

The writer, in 1892, obtained the following results in experiments with naphthols upon the cholera spirillum.

Alpha-naphthol and beta-naphthol have about the same antiseptic and germicidal value. In the proportion of 1 : 16,000 both prevent the development of the cholera spirillum in peptonized beef-tea, while 1 : 24,000 fails to prevent development. In the proportion of 1 : 3,000 both destroy the vitality of the cholera spirillum in bouillon cultures, twenty-four hours old, after two hours' contact, while 1 : 4,000 fails to destroy this microörganism in the time mentioned—two hours.

In experiments made with a solution of 1 : 1,000, added to an equal quantity of a twenty-four hours old bouillon culture—making 1 : 2,000 after mixture—and in which the time of contact varied from five to thirty minutes, alpha-, beta-, and hydronaphthol were found to destroy the cholera germ by fifteen minutes' exposure, but to fail after ten minutes' contact, so that the germicidal value of each of these is similar, or nearly so.

In all these experiments the line was sharply drawn between success and failure. No development occurred and the bouillon remained transparent in those experiments in which the germicidal action was complete, and a characteristic development occurred within twenty-four hours in those experiments in which there was a failure to destroy the spirillum.

Benzo-naphthol has no germicidal power, probably because it is insoluble in water. At least this is my inference from the experiments made. One gamme was added to one thousand cubic centimetres of distilled water, and after vigorous shaking was placed in the steam sterilizer for half an hour. At the end of this time the greater portion, at least, of the benzo-naphthol remained undissolved at the bottom of the flask. The saturated solution (?) was then filtered and added to recent bouillon cultures of the cholera spirillum in the proportion of 1 : 1, 1 : 2, 1 : 4, and 2 : 1. At the end of two hours sterile bouillon in test tubes was inoculated from each of these and placed in the incubating oven. At the end of forty-eight hours a characteristic development of the cholera spirillum had occurred in all of the tubes.

Olive Oil.—Anthrax spores germinate after having been immersed for ninety days in pure olive oil (Koch).

Oil of Mustard.—Koch found that the development of anthrax spores is prevented by 1 : 33,000.

Oil of Peppermint.—A five-per-cent solution in alcohol failed in twelve days to destroy anthrax spores, but the development of these spores is restrained by 1 : 33,000 (Koch).

Oil of Turpentine destroys anthrax spores in five days, but failed to do so in one day (Koch). The development of anthrax spores is prevented by 1 : 75,000 (Koch). The addition of 1 : 200 to nutrient gelatin prevents the development of bacteria (Riedlin). An excess of oil of turpentine added to a liquefied gelatin culture of Staphylococcus aureus does not destroy this micrococcus in five hours (v. Christmas-Dirckinck-Holmfeld).

Saprol.—Laser (1892) recommends this agent for the disinfection of the excreta of cholera and typhoid patients. He reports that in the proportion of 1 : 100 it sterilizes liquid fæces in twenty-four hours.

Skatol in excess in water has no germicidal power, as tested upon anthrax spores (Koch).

Smoke.—The researches of Beu show that meats which have been preserved by smoking commonly contain living bacteria capable of growing in culture media; and Petri has shown that pork which has been salted for a month and then smoked for fourteen days may still contain the bacillus of rothlauf in a living condition, as shown by inoculation experiments. It was not until about six months after smoking that the bacillus failed to give evidence of vitality.

Thymol.—A five-per-cent solution in alcohol does not destroy anthrax spores in fifteen days, but the development of these spores is retarded by a solution of 1 : 80,000 (Koch). The anthrax bacillus and staphylococci fail to grow in culture media containing 1 : 3,000 (Samter). The tubercle bacillus is destroyed by contact with thymol for three hours (Yersin). Thymol has about four times less germicidal power than carbolic acid (Behring). Antiseptic in the proportion of 1 : 1,340 (Miquel).

Tobacco Smoke.—Tassinari found that tobacco smoke restrains the development of bacteria, and that certain species failed to develop after exposure for half an hour in an atmosphere of tobacco smoke—spirillum of cholera and Friedländer's bacillus.

XII.

ACTION OF BLOOD SERUM AND OTHER ORGANIC LIQUIDS.

Blood Serum.—Bacteriologists have long been aware of the fact that many species of bacteria, when injected into the circulation of a living animal, soon disappear from the blood, and that the blood of such an animal a few hours after an injection of putrefactive bacteria, for example, does not contain living bacteria capable of developing in a suitable nutrient medium. Wyssokowitsch, in an extended series of experiments, has shown that non-pathogenic bacteria injected into the circulation may be obtained in cultures from the liver, spleen, kidneys, and bone marrow after they have disappeared from the blood, but that, as a rule, those present in these organs have lost their vitality, as shown by culture experiments, in a period varying from a few hours to two or three days. According to the theory of Metschnikoff, this destruction of bacteria in the blood and tissues of a living animal is effected by the cellular elements, and especially by the leucocytes, which pick up and digest these vegetable cells very much as an amœba disposes of similar microörganisms which serve it as food. Some such theory seemed necessary to account for the disappearance of bacteria from the blood before the demonstration was made that the serum of the circulating fluid, quite independently of its cellular elements, possesses very decided germicidal power.

Von Fodor first (1887) called attention to the fact that anthrax bacilli may be destroyed by freshly drawn blood; and Nuttall (1888), in an extended series of experiments, showed that various bacteria are destroyed within a short time by the fresh blood of warm-blooded animals. Thus the anthrax bacillus in rabbit's blood was usually killed in from two to four hours when the temperature was maintained at 37°–38° C., and the same result was obtained with pigeon's blood at 41° C. But when the blood was allowed to stand for a considerable time, or was heated for forty-five minutes to 45° C., it served as a culture fluid, and an abundant development of anthrax bacilli occurred in it. Bacillus subtilis and Bacillus megatherium were also destroyed in two hours by fresh rabbit's blood, but it was without action on Staphylococcus pyogenes aureus, which at a temperature of 37.5° C. was found to have increased in numbers at the end of two hours. Further researches by Nissen and Behring show that there is a wide difference in the blood of different animals as to germicidal power, and that certain bacteria are promptly destroyed, while other species are simply restrained for a time in their development or are not affected. Thus Nissen found that the cholera spirillum, the bacillus of anthrax, the bacillus of typhoid fever, and Friedländer's pneumococcus were killed, while Staphylococcus pyogenes aureus and albus, the streptococcus of erysipelas, the bacillus of fowl cholera, the bacillus of rothlauf, and Proteus hominis were able to multiply in rabbit's blood after having been restrained for a short time in their development. In the case of the cholera spirillum a period of ten to forty minutes sufficed for the complete destruction of a limited number, but when the number exceeded 1,200,000 per cubic centimetre they were no longer destroyed with certainty, and after five hours an increase occurred. The anthrax bacillus was commonly destroyed within twenty minutes and the typhoid bacillus at the end of two hours. In the experiments of Behring and Nissen it was found that the most pronounced germicidal effect upon the anthrax bacillus was obtained from the blood of the rat, an animal which has a natural immunity against anthrax; while the blood of the guinea-pig, a very susceptible animal, had no restraining effect and served as a favorable culture medium for the anthrax bacillus. And the remarkable fact was demonstrated that when the blood of a rat was added to the blood of the guinea-pig in the proportion of 1 : 8, it exercised a decided restraining influence upon the growth of the anthrax bacillus. Later researches have shown that cultivation in the blood of an immune animal causes an attenuation of the virulence of an anthrax culture (Ogata and Jasuhara); and also that the injection of the blood of a frog or rat—naturally immune—into a susceptible animal which has been inoculated with a virulent culture of the anthrax bacillus, will prevent the death of the inoculated animal.

Buchner has shown that the germicidal power of the blood of dogs and rabbits does not depend upon the presence of the cellular elements, but is present in clear serum which has been allowed to separate from the clot in a cool place. Exposure for an hour to a temperature of 55° C. destroys the germicidal action of serum as well as of blood; the same effect is produced by heating to 52° C. for six hours or to 45.6° C. for twenty hours. The germicidal power of blood serum is not destroyed by freezing and thawing, but is lost after it has been kept for some time. Buchner's experiments led him to the conclusion that the germicidal power of fresh blood serum depends upon the presence of some albuminous body present in it. This view is sustained by the researches of Ogata, who has obtained from the blood of dogs and other animals a glycerin extract of a "ferment" which is insoluble in alcohol or in ether and which has germicidal properties.

According to Emmerich and Tsuboi (1893), when the serum-albumin is precipitated by alcohol, dried in a vacuum at 40° C., and dissolved in water it has no longer any germicidal activity. But if the precipitated and dried albumin is dissolved at 39° C. in a weak solution (0.05-0.08 per cent) of soda or potash it recovers its original germicidal value.

It has been demonstrated by several experimenters that *other albuminous fluids* possess a similar germicidal power. Thus Nuttall found that a pleuritic exudation from man destroyed the anthrax bacillus in an hour, the aqueous humor of a rabbit in two hours. Wurz has experimented with fresh egg albumin, and found that the anthrax bacillus failed to grow after having been exposed for an hour to the action of albumin from a hen's egg; other bacteria tested were not killed so promptly, but a decided germicidal action was manifested. Prudden has shown that the albuminous fluid obtained from a hydrocele, or from the abdominal cavity in ascites, possesses similar germicidal power; and Fokker has demonstrated that *fresh milk* destroys the vitality of certain bacteria which induce an acid fermentation of this fluid.

The results heretofore referred to induced Hankin to experiment with *cell globulin* obtained from the spleen or lymphatic glands of a dog or cat. This is extracted by means of a solution of chloride of sodium, the solution is filtered, and the globulin precipitated by the addition of alcohol. The precipitate is washed and again dissolved in salt solution. The result showed that this cell globulin possesses germicidal power similar to that of blood serum.

Mucus.—The experiments of Wurtz and Lermoyez (1893) show that nasal mucus has germicidal properties, especially for the anthrax bacillus. Walthard (1893), in experiments with mucus from the cervix uteri, was not able to demonstrate any germicidal action, but arrived at the conclusion that it prevents the development of bacteria simply because it is an unfavorable medium. Various bacteria were planted upon the surface of cervical mucus in Petri dishes, and placed in the incubating oven, but all failed to grow.

Nucleins from animal and vegetable cells have been shown by Professor Vaughan and his associates (1893) to possess considerable germicidal power. The nucleins of animal origin were obtained from the testes of dogs and rats. Dissolved in a 0.5-per-cent solution of caustic potash and then diluted with four volumes of physiologic salt solution the germicidal activity was shown by the facts that Staphylococcus pyogenes aureus, and the anthrax bacillus without spores, failed to grow after twenty minutes' exposure. Kossel (1893) has obtained similar results with nucleins from the thymus gland of the calf.

Urine.—The experiments of Lehmann show that fresh urine has a decided germicidal power for the cholera spirillum and the anthrax bacillus, and no doubt for other bacteria as well. To what constituent of the urine this is due has not been determined, but it may be due to the uric acid present.

XIII.

PRACTICAL DIRECTIONS FOR DISINFECTION.

THE Committee on Disinfectants of the American Public Health Association (appointed in 1884), after an extended investigation with reference to the germicidal value of various agents, in a final report submitted in 1887 submits the following "Conclusions":

The experimental evidence recorded in this report seems to justify the following conclusions:

The most useful agents for the destruction of spore-containing infectious material are—

1. *Fire.* Complete destruction by burning.
2. *Steam under pressure.* 105° C. (221° F.) for ten minutes.
3. *Boiling in water* for half an hour.
4. *Chloride of lime.*[1] A four-per-cent solution.
5. *Mercuric chloride.* A solution of 1:500.

For the destruction of infectious material which owes its infecting power to the presence of microörganisms not containing spores, the committee recommends—

1. *Fire.* Complete destruction by burning.
2. *Boiling in water* for ten minutes.
3. *Dry heat.* 110° C. (230° F.) for two hours.
4. *Chloride of lime.* A two-per-cent solution.
5. *Solution of chlorinated soda.*[2] A ten-per-cent solution.
6. *Mercuric chloride.* A solution of 1:2,000.
7. *Carbolic acid.* A five-per-cent solution.
8. *Sulphate of copper.* A five-per-cent solution.
9. *Chloride of zinc.* A ten-per-cent solution.
10. *Sulphur dioxide.*[3] Exposure for twelve hours to an atmosphere containing at least four volumes per cent of this gas in presence of moisture.

The committee would make the following recommendations with reference to the practical application of these agents for disinfecting purposes:

FOR EXCRETA.

(*a*) In the sick-room:

1. Chloride of lime in solution, four per cent.

In the absence of spores:

2. Carbolic acid in solution, five per cent.
3. Sulphate of copper in solution, five per cent.

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[1] Should contain at least twenty-five per cent of available chlorine.

[2] Should contain at least three per cent of available chlorine.

[3] This will require the combustion of between three and four pounds of sulphur for every thousand cubic feet of air space.

(*b*) In privy vaults:
1. Mercuric chloride in solution, 1:500.[1]
2. Carbolic acid in solution, five per cent.

(*c*) For the disinfection and deodorization of the surface of masses of organic material in privy vaults, etc.:
Chloride of lime in powder.

FOR CLOTHING, BEDDING, ETC.

(*a*) Soiled underclothing, bed linen, etc.:
1. Destruction by fire, if of little value.
2. Boiling for at least half an hour.
3. Immersion in a solution of mercuric chloride of the strength of 1:2,000 for four hours.
4. Immersion in a two-per-cent solution of carbolic acid for four hours.

(*b*) Outer garments of wool or silk, and similar articles, which would be injured by immersion in boiling water or in a disinfecting solution:
1. Exposure in a suitable apparatus to a current of steam for ten minutes.
2. Exposure to dry heat at a temperature of 110° C. (230° F.) for two hours.

(*c*) Mattresses and blankets soiled by the discharges of the sick:
1. Destruction by fire.
2. Exposure to superheated steam, 105° C. (221° F.), for ten minutes. (Mattresses to have the cover removed or freely opened.)
3. Immersion in boiling water for half an hour.

FURNITURE AND ARTICLES OF WOOD, LEATHER, AND PORCELAIN.

Washing, several times repeated, with—
1. Solution of carbolic acid, two per cent.

FOR THE PERSON.

The hands and general surface of the body of attendants of the sick, and of convalescents, should be washed with—
1. Solution of chlorinated soda diluted with nine parts of water, 1:10.
2. Carbolic acid, two-per-cent solution.
3. Mercuric chloride, 1:1,000.

FOR THE DEAD.

Envelop the body in a sheet thoroughly saturated with—
1. Chloride of lime in solution, four per cent.
2. Mercuric chloride in solution, 1:500.
3. Carbolic acid in solution, five per cent.

FOR THE SICK-ROOM AND HOSPITAL WARDS.

(*a*) While occupied, wash all surfaces with—
1. Mercuric chloride in solution, 1:1,000.
2. Carbolic acid in solution, two per cent.

(*b*) When vacated, fumigate with sulphur dioxide for twelve hours, burning at least three pounds of sulphur for every thousand cubic feet of air space in the room; then wash all surfaces with one of the above-mentioned disinfecting solutions, and afterward with soap and hot water; finally throw open doors and windows, and ventilate freely.

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[1] The addition of an equal quantity of potassium permanganate as a deodorant, and to give color to the solution, is to be recommended. [The writer no longer indorses this recommendation. See his paper on "The Disinfection of Excreta," appended.]

FOR MERCHANDISE AND THE MAILS.

The disinfection of merchandise and of the mails will only be required under exceptional circumstances; free aëration will usually be sufficient. If disinfection seems necessary, fumigation with sulphur dioxide will be the only practicable method of accomplishing it without injury.

RAGS.

(*a*) Rags which have been used for wiping away infectious discharges should at once be burned.

(*b*) Rags collected for the paper-makers during the prevalence of an epidemic should be disinfected, before they are compressed in bales, by—

1. Exposure to superheated steam of 105° C. (221° F.) for ten minutes.
2. Immersion in boiling water for half an hour.

SHIPS.

(*a*) Infected ships at sea should be washed in every accessible place, and especially the localities occupied by the sick, with—

1. Solution of mercuric chloride, 1:1,000.
2. Solution of carbolic acid, two per cent.

The bilge should be disinfected by the liberal use of a strong solution of mercuric chloride.

(*b*) Upon arrival at a quarantine station, an infected ship should at once be fumigated with sulphurous acid gas, using three pounds of sulphur for every thousand cubic feet of air space; the cargo should then be discharged on lighters; a liberal supply of the concentrated solution of mercuric chloride (four ounces to the gallon) should be thrown into the bilge, and at the end of twenty-four hours the bilge water should be pumped out and replaced with pure sea water; this should be repeated. A second fumigation, after the removal of the cargo, is recommended; all accessible surfaces should be washed with one of the disinfecting solutions heretofore recommended, and subsequently with soap and hot water.

FOR RAILWAY CARS.

The directions given for the disinfection of dwellings, hospital wards, and ships apply as well to infected railway cars. The treatment of excreta with a disinfectant, before they are scattered along the tracks, seems desirable at all times in view of the fact that they may contain infectious germs. During the prevalence of an epidemic of cholera this is imperative. For this purpose the standard solution of chloride of lime is recommended.

DISINFECTION BY STEAM.

The Committee on Disinfectants, in the above-quoted "Conclusions," recommends the use of "steam under pressure, 105° C. (221° F.), for ten minutes" for the destruction of spore-containing infectious material. The spores of all known pathogenic bacteria are destroyed by a temperature of 100° C. maintained for five minutes, and in view of this fact the temperature fixed by the committee is ample, and to exact a higher temperature or longer exposure would be unreasonable. But in practical disinfection the temperature required to destroy infectious material is not the only question to be considered. Economy in the construction and operation of the steam disinfecting apparatus must have due attention, and an important point relates to the penetration of porous, non-conducting articles, such as rolls of blankets, clothing, etc. These points have been the subject of numerous experimental investigations, and the principles involved have been elucidated, especially by the investigations of Esmarch (1887), of Budde (1889), and of Teuschner (1890).

It has been shown that streaming steam is more effective than confined steam at the same temperature, because it penetrates porous objects more quickly. Also that superheated, " dry " steam is not as effective as flowing steam at 100° C.; on the other hand, it corresponds in effectiveness with dry air, and the temperature must be raised to 140° to 150° C. in order to quickly destroy the spores of bacilli.

Esmarch's investigations show that streaming steam penetrates porous objects, like rolled blankets, more readily than confined steam; but the later researches of Budde and of Teuschner show that a temperature of 100° C. is more rapidly reached in the interior of such rolls when the flowing steam is under pressure. With the same pressure (fifteen pounds) a temperature of 100° C. was reached in two and one-half minutes when the steam was flowing, and in eleven minutes by steam at rest (Budde). Intermittent pressure was not found by Budde to present any advantages over continuously flowing steam; on the contrary, the time of penetration was longer.

Teuschner, whose investigations are the most recent, arrives at the following conclusions:

1. Strongly superheated steam is not to be recommended for practical disinfection. On the contrary, a slight superheating of the steam, such as occurs in the apparatus of Schimmel, is not objectionable.
2. Those forms of apparatus in which the steam enters from above are much safer and quicker in their disinfecting action than those in which this is not the case. In the construction of such apparatus care must be taken, in order to secure penetration of the objects, that the air and steam have a free escape below.
3. Disinfection is hastened by previously warming the apparatus.
4. The most rapid disinfecting action is secured by the use of streaming steam in a state of tension (under pressure).
5. Objects which have been in contact with fatty or oily substances require a longer time for disinfection than those which have not.
6. To accomplish disinfection it is necessary to expel, as completely as possible, all air from the objects to be disinfected, and also to secure a sufficient condensation of the steam.
7. The condensation of the steam advances in a sharply defined line from the periphery to the centre of porous objects.
8. The temperature necessary for disinfection is only found in the zone where condensation has already taken place.
9. Only a few centimetres from the zone in which the temperature is 100° C.—when disinfection is incomplete—there may be places in which the temperature is 40° C. or more below the boiling point.

DISINFECTION BY FORMALDEHYDE GAS.

Recent experiments have demonstrated the valuable germicidal properties of formaldehyde gas. Owing to its superior germicidal value and non-toxic properties, it has to a considerable extent taken the place of sulphur dioxide as a gaseous disinfectant. In making practical use of this agent a suitable apparatus will be required. For the disinfection of a room with its contents, freely exposed for surface disinfection, one pound of formalin should be volatilized for each thousand cubic feet of air space—the time of exposure to the disinfecting action of the gas being not less than twelve hours. When paraform is used the amount required will be sixty grammes to one thousand cubic feet (Novy). In the absence of any apparatus satisfactory results have been obtained by the Department of Health of the city of Chicago, as follows:

> "Ordinary bed sheets were employed to secure an adequate evaporatory surface, and these, suspended in the room, were simply sprayed with a forty-per-cent solution of formalin through a common watering-pot rose-head. A sheet of the usual size and quality will carry from one hundred and fifty to one hundred and eighty cubic centimetres of the solution without dripping, and this quantity has been found sufficient for the disinfection of one thousand cubic feet of space. Of course, the sheets may be modified to any necessary number. . . . Surface disinfection was thorough, while a much greater degree of penetration was shown than that secured by any other method."

Formalin may also be used in the disinfection of rooms and their contents by spraying all exposed surfaces.

Experiments made by Kinyoun and others show that formaldehyde gas does not injure the color or textile strength of fabrics of wool, silk, cotton, or linen, and that it has no injurious action upon furs, leather, copper, brass, nickel, zinc, polished steel or gilt work. Iron and unpolished steel are attacked by the gas.

DISINFECTION OF THE HANDS.

The importance of a reliable method of disinfecting the hands of surgeons, obstetricians, and nurses after they have been in contact with infectious material from wounds, puerperal discharges, etc., is now fully recognized, and some surgeons consider it necessary to completely sterilize the hands before undertaking any surgical operation which will bring them in contact with the freshly-cut tissues. The numerous experiments which have been made with a view to ascertaining the best method of accomplishing such sterilization of the hands show that it is by no means a simple matter to effect it, and especially to insure the destruction of microörganisms concealed beneath the finger nails. Fürbringer, in an extended series of experiments (1888), found that a preliminary cleansing with soap and a brush was even more important than the degree of potency of the disinfecting wash subsequently applied. He recommends the following procedure :

1. Remove all visible dirt from beneath and around the nails.

2. Brush the spaces beneath the nails with soap and hot water for a minute.

3. Wash for a minute in alcohol (not below eighty per cent), and, before this evaporates, in the following solution :

4. Wash thoroughly for a minute in a solution containing 1 : 500 of mercuric chloride or three per cent of carbolic acid.

Roux and Reynés tested the above method of Fürbringer, and found that it gave better results than others previously proposed, although not always entirely successful in securing complete sterilization.

Boll has recently (1890) reported favorable results from the following method :

1. Cleanse the finger nails from visible dirt with knife or nail scissors.
2. Brush the hands for three minutes with hot water and potash soap.
3. Wash for half a minute in a three-per-cent solution of carbolic acid, and subsequently in a 1 : 2,000 solution of mercuric chloride.
4. Rub the spaces beneath the nails and around their margins with iodoform gauze wet in a five-per-cent solution of carbolic acid.

Welch, as a result of extended experiments made at the Johns Hopkins Hospital, recommends the following procedure :

1. The nails are kept short and clean.
2. The hands are washed thoroughly for several minutes with soap and water, the water being as warm as can be comfortably borne, and being frequently changed. A brush sterilized by steam is used. The excess of soap is washed off with water.
3. The hands are immersed for one or two minutes in a warm saturated solution of permanganate of potash and are rubbed over thoroughly with a sterilized swab.
4. They are then placed in a warm saturated solution of oxalic acid, where they remain until complete decolorization of the permanganate occurs.
5. They are then washed off with sterilized salt solution or water.
6. They are immersed for two minutes in sublimate solution, 1 : 500.

The bacteriological examination of the skin thus treated yields almost uniformly negative results, the material for the cultures being taken from underneath and around the nails. This is the procedure now employed in the gynecological and surgical wards of the hospital.

THE DISINFECTION OF EXCRETA.

The contents of privy vaults and cesspools should never be allowed to accumulate unduly or to become offensive. By frequent removal, and by the liberal use of antiseptics, such necessary receptacles of filth should be kept in a sanitary condition. The absorbent deodorants, such as dry earth or pounded charcoal, or the chemical deodorants and antiseptics, such as chloride of zinc, sulphate of iron, etc., will, under ordinary circumstances, prevent such places from becoming offensive. Disinfection will be required only when it is known or suspected that infectious material, such as the dejections of patients with cholora, yellow fever, or typhoid fever, has been thrown into the receptacles.

In the Manual for the Medical Department of the United States Army the following directions are given:

> 92. When accumulations of organic material undergoing decomposition cannot be removed or buried, they may be treated with an antiseptic solution, or with freshly burned quicklime. Quicklime is also a valuable disinfectant, and may be substituted for the more expensive chloride of lime for disinfection of typhoid and cholera excreta, etc. For this purpose freshly prepared *milk of lime* should be used, containing about one part, by weight, of hydrate of lime, to eight of water.
>
> 93. During the prevalence of an epidemic, or when there is reason to believe that infectious material has been introduced from any source, latrines and cesspools may be treated with milk of lime, in the proportion of five parts to one hundred parts of the contents of the vault, and the daily addition of ten parts for one hundred parts of daily increment of fæces.

According to Behring, lime has about the same germicidal value as the other caustic alkalies, and destroys the cholera spirillum and the bacillus of typhoid fever, of diphtheria, and of glanders after several hours' exposure, in the proportion of fifty cubic centimetres *normal-lauge* per litre. Wood ashes or lye of the same alkaline strength may therefore be substituted for quicklime.

Finally, it must not be forgotten that we have a ready means of disinfecting excreta in the sick-room or its vicinity by the application of heat. Exact experiments, made by the writer and others, show that the thermal death-point of the following pathogenic bacteria, and of the kinds of virus mentioned, is below 60° C. (140° F.): Spirillum of cholera, bacillus of anthrax, bacillus of typhoid fever, bacillus of diphtheria, bacillus of glanders, diplococcus of pneumonia (Micrococcus Pasteuri), streptococcus of erysipelas, staphylococci of pus, micrococcus of gonorrhœa, vaccine virus, sheep-pox virus, hydrophobia virus. Ten minutes' exposure to the temperature mentioned may be relied upon for the disinfection of material containing any of these pathogenic organisms, except the anthrax bacillus when in the stage of spore formation. The use, therefore, *of boiling water in the proportion of three or four parts to one part of the material to be disinfected* may be safely recommended for such material. Or, better still, a ten-per-cent solution of sulphate of iron or of chloride of zinc at the boiling-point may be used in the same way (three parts to one).

PART THIRD.

PATHOGENIC BACTERIA.

I. Modes of Action. II. Channels of Infection. III. Susceptibility and Immunity. IV. Protective Inoculations. V. Pyogenic Bacteria. VI. Bacteria in Croupous Pneumonia. VII. Pathogenic Micrococci not described in Sections V. and VI. VIII. The Bacillus of Anthrax. IX. The Bacillus of Typhoid Fever. X. Bacteria in Diphtheria. XI. Bacillus of Influenza. XII. Bacilli in Chronic Infectious Diseases. XIII. Bacilli which produce Septicæmia in Susceptible Animals. XIV. Pathogenic Aërobic Bacilli not described in Previous Sections. XV. Bacteria of Plant Diseases. XVI. Pathogenic Anaërobic Bacilli. XVII. Pathogenic Spirilla.

PART THIRD.

PATHOGENIC BACTERIA.

I.

MODES OF ACTION.

MANY of the saprophytic bacteria are pathogenic for man, or for one or more species of the lower animals, when by accident or experimental inoculation they obtain access to the body ; these may be designated *facultative parasites*. Other species which, for a time at least, are able to lead a saprophytic mode of life have their normal habitat in the bodies of infected animals, in which they produce specific infectious diseases. To this class belong the cholera spirillum, the anthrax bacillus, the bacillus of typhoid fever, and various other microörganisms which are the cause of specific infectious diseases in some of the lower animals. These we may speak of as *parasites* and *facultative saprophytes*. Still others are *strict parasites* and do not find the conditions for their development outside of the bodies of the animals which they infest, except under the special conditions in which bacteriologists have succeeded in cultivating some of them. The best known strict parasites are the tubercle bacillus, the bacillus of leprosy, the spirillum of relapsing fever, and the micrococcus of gonorrhœa.

There can be but little doubt that even the strict parasites, at some time in the past, were also saprophytes, and that the adaptation to a parasitic mode of life was gradually effected under the laws of natural selection. In a previous chapter (Section III., Part Second) we have referred to the modifications in biological characters which may occur as a result of special conditions of environment. Thus we may obtain non-chromogenic varieties of species which usually produce pigment, or non-pathogenic varieties of bacteria which are usually pathogenic. There is also evidence that the tubercle bacillus, a strict parasite, may be so modified, by cultivation for successive generations in a culture medium containing glycerin, that it will finally grow in ordinary beef infusion, thus showing a tendency to adapt itself to a saprophytic mode of life.

Some of the saprophytic bacteria are indirectly pathogenic by reason of their power to multiply in articles of food, such as milk, cheese, fish, sausage, etc., and there produce poisonous ptomaines which, when these articles are ingested, give rise to various morbid symptoms, such as vomiting, gastric and intestinal irritation, fever, etc. Or similar symptoms may result from the multiplication of *bacteria producing toxic ptomaines in the alimentary canal.* No doubt gastric and intestinal disorders are largely due to this cause, and may be induced by a variety of saprophytic bacteria when these establish themselves in undue numbers in any portion of the alimentary tract. In Asiatic cholera the same thing occurs, but with more fatal results from the introduction of the East Indian cholera germ discovered by Koch. This is pathogenic for man, because it is able to multiply rapidly in the human intestine, and there produces a toxic substance which, being absorbed, gives rise to the morbid phenomena of the disease. The spirillum itself does not enter the blood or invade the tissues, except to a limited extent in the mucous coat of the intestine, and the true explanation of its pathogenic power is no doubt that which has been given.

Other microörganisms invade the tissues and multiply in certain favorable localities, but have not the power of developing in the blood, in which they are only found occasionally and in very small numbers or not at all. Thus the typhoid bacillus locates itself in the intestinal glands, in the spleen, and in the liver, forming colonies of limited extent, and evidently not finding the conditions extremely favorable for its growth, inasmuch as it does not take complete possession of these organs. The symptoms which result from its presence are doubtless partly due to local irritation, disturbance of function, and, in the case of the intestinal glands, necrotic changes induced by it. But in addition to this its pathogenic action depends upon the production of a poisonous ptomaine which has been isolated and studied by the German chemist Brieger (typhotoxin).

Certain saprophytic bacteria, when injected beneath the skin of a susceptible animal, multiply at the point of inoculation and invade the surrounding tissues, giving rise in some instances to the formation of a local abscess, in others to an infiltration of the tissues with bloody serum, and in others to extensive necrotic changes. These local changes are due not simply to the mechanical presence of the microörganisms which induce them, but to chemical products evolved during the growth of these pathogenic bacteria. Indeed, their pathogenic power evidently depends, in some instances at least, upon these toxic products of their growth, by which the vital resisting power of the tissues is overcome.

Among the bacteria which in this way produce extensive local inflammatory and necrotic changes are certain anaërobic species found in the soil and in putrefying material, such as the bacillus of malignant œdema and the writer's Bacillus cadaveris. The bacillus of symptomatic anthrax, an infectious disease of cattle, acts in the same way. All of these produce toxic substances which have a very pronounced local action upon the tissues invaded by them. Other bacteria, while they develop chiefly in the vicinity of the point of entrance—by accident or by inoculation—produce a potent toxic substance which gives rise to general symptoms of a serious character, such as tetanic convulsions (bacillus of tetanus) or intense fever and nervous phenomena (micrococcus of erysipelas). Again, the local irritation resulting from the presence of parasitic bacteria may primarily give rise to the formation of new growths having a low grade of vitality, which later may undergo necrotic changes, as in tuberculosis, glanders, and leprosy. In this case constitutional symptoms are not present, or are of a mild character during the development of these new formations, which apparently result from the local action of substances eliminated during the growth of the parasite, rather than from its simple presence. This is an inference based upon the fact that non-living particles, or even living parasites, as in trichinosis, do not produce similar new growths composed of cells, but become encysted in a fibrous capsule.

In pneumonia we have a local process in which one or more lobes of the lung are invaded by a pathogenic micrococcus (Micrococcus pneumoniæ crouposæ) which induces a fibrinous exudation that completely fills the air cells. How far the symptoms of the disease are due to the local inflammation and disturbance of function, and to what extent they may be due to the absorption of a soluble toxic substance evolved as a result of the growth of the micrococcus, has not been determined. But the mild character of the general symptoms when a limited area of lung tissue is involved leads to the inference that the pathogenic power of this particular pathogenic microörganism is chiefly exercised locally.

The pus cocci and various other saprophytic bacteria, when introduced beneath the skin, give rise to the *formation of abscesses,* unattended by any very considerable general disturbance; and also to secondary purulent accumulations—metastatic abscesses.

That this is not due simply to their mechanical presence is shown by the fact that powdered glass and other inert substances, when thoroughly sterilized, do not give rise to pus formation when introduced beneath the skin or injected into the cavity of the abdomen. On the other hand, it has been demonstrated by the experiments of Grawitz, De Bary, and others that certain chemical substances which act as local irritants when brought in contact with the tissues may induce pus formation quite independently of microörganisms: nitrate of silver, oil of turpentine, and strong liquor ammoniæ have been shown to possess this power. And it has been demonstrated by the recent experiments of Buchner that sterilized cultures of a long list of different bacteria—seventeen species tested—give rise to suppuration when introduced into the subcutaneous tissues.

Buchner has further shown that this property of inducing pus formation resides in the dead bacterial cells and not in soluble products present in the cultures. For the clear fluid obtained by passing these sterilized cultures through a porcelain filter gave a negative result, while the bacteria retained by the filter, although no longer capable of development, having been killed by heat, invariably caused suppuration.

Individuals suffering from malnutrition are more susceptible to invasion by specific disease germs or by the common pus cocci than are those in vigorous health. Thus the sufferers from starvation, from crowd poisoning, sewer-gas poisoning, etc., are not only liable to be early victims during the prevalence of an epidemic disease, but are very subject to abscesses, boils, ulcers, etc. A slight abrasion in such an individual, inoculated by the ever-present pus cocci, may give rise to an obstinate ulcer or a phlegmonous inflammation.

In the same way some of the ordinary saprophytes, which usually have no pathogenic power, may be pathogenic for an animal whose strength is reduced by disease or injury. Thus necrotic changes may occur in injured tissues, or in those which have a deficient blood supply—from occlusion of an artery, for example—due to the presence of putrefactive bacteria which are incapable of development in the circulation of a healthy animal or in healthy tissues. We may also have a *progressive gangrene*, due to infection of wounds by bacteria which are able to invade healthy tissues. This is seen in the so-called hospital gangrene, which is undoubtedly due to microörganisms, although the species concerned in its production has not been determined, owing to the fact that modern bacteriologists have had few, if any, opportunities for studying it. The history of the disease, its rapid extension in infected surgical wards, the extensive sloughing which occurs within a few hours in previously healthy wounds, and the effect of deep cauterization by the hot iron, nitric acid, or bromine in arresting the progress of the disease, all support this view of its etiology. Whether it is due to a specific pathogenic micro-organism, or to exceptional pathogenic power acquired by some one of the common bacteria which infest suppurating wounds, cannot be determined in the absence of exact experiments by modern methods. But the latter view has seemed to the writer the most probably correct. There are many facts which go to show that pathogenic virulence may be increased by cultivation in animal fluids, and where wounded men are brought together under unfavorable sanitary conditions, as has been the case where hospital gangrene has made its appearance, it may be that some common saprophyte acquires the power of invading the exposed tissues instead of simply feeding upon the secretions which bathe its surface.

Koch has described a progressive tissue necrosis in mice, due to a streptococcus, which he first obtained by inoculating a mouse in the ear with putrid material. The morbid process is entirely local and rapidly progressive, causing a fatal termination in about three days, without invasion of the blood.

In *diphtheritic inflammations* of mucous membranes we have a local invasion of the tissues and a characteristic plastic exudation. In true diphtheria the local inflammation and necrotic changes in the invaded tissues are not sufficient to account for the serious general symptoms, and we now have experimental evidence that the diphtheria bacillus produces a very potent toxic substance to which these symptoms are no doubt largely due. The diphtheria bacillus of Löffler appears to be the cause of the fatal malady which goes by this name, but undoubtedly other microörganisms may be concerned in the formation of diphtheritic false membranes. In certain forms of diphtheria, and especially when it occurs as a complication of scarlet fever, measles, and other diseases, the Klebs-Löffler bacillus is absent, and a streptococcus, which appears to be identical with Streptococcus pyogenes, is found in considerable numbers and is probably the cause of the diphtheritic inflammation. An epidemic of diphtheria occurring among calves was studied by Löffler, and is ascribed by him to his Bacillus diphtheriæ vitulorum. The same bacteriologist has shown that the diphtheria of chickens and of pigeons is due to a specific bacillus which differs from that found in human diphtheria, and which he calls Bacillus diphtheriæ columbrarum.

Prof. Welch has studied the histological lesions produced by filtered cultures of the diphtheria bacillus. Cultures in glycerin-bouillon, several weeks old, were filtered through porcelain, and the sterile filtrate was injected beneath the skin of guinea-pigs. One cubic centimetre of this filtrate was injected into a guinea-pig on the 10th of December, and two cubic centimetres more on the 14th of the same month. The animal succumbed at the end of three weeks and five days after the first inoculation. At the autopsy "the lymphatic glands of the inguinal and axillary regions were found to be enlarged and reddened; the cervical glands were swollen and the thyroid gland was greatly congested. There was a considerable excess of clear fluid in the peritoneal cavity. Both layers of the peritoneum were reddened, the vessels of the visceral layer being especially injected. The spleen was enlarged to double the average size; it was mottled, and the white follicles were distinctly outlined against the red ground. The liver was dark in color and contained much blood. . . . The kidneys were congested and the cut surface was cloudy. . . . The pericardial sac was distended with clear serum. Under the epicardium were many ecchymotic spots. The lungs exhibited areas of intense congestion or actual hæmorrhage into the tissues. . . . The histological lesions in this case are identical with those observed by us in connection with the inoculation of the living organisms."

To what extent non-specific *catarrhal inflammations* of mucous membranes are caused by the local action of microörganisms has not been determined, but in gonorrhœa the proof is now considered satisfactory that the "gonococcus" of Neisser is the cause of the intense local inflammation and purulent discharge. In this disease the action of the pathogenic microörganism seems to be limited to the tissues invaded by it, as there is no general systemic disturbance indicating the absorption of a toxic ptomaine.

Chronic catarrhal inflammations appear, in some cases at least, to be kept up by the presence of microörganisms, which are always found in the discharges from inflamed mucous surfaces.

The influence of microörganisms, and especially of the pus cocci, in preventing the prompt healing of wounds, is now well established. An extensive suppurating wound or collection of pus, especially if putrefactive bacteria are present, causes fever and nervous symptoms, due to the absorption of toxic products. More intense general symptoms result from the presence of the streptococcus of pus than from the less pathogenic staphylococci; this is seen in erysipelatous inflammations and in puerperal metritis due to the presence of this micrococcus. Like the other pus cocci, the Streptococcus pyogenes does not usually invade the blood, but when introduced into the subcutaneous tissues it induces a local inflammatory process, with a tendency to pus formation, and it invades the neighboring lymph channels, in which the conditions appear to be especially favorable for its multiplication.

Finally, certain pathogenic bacteria, when introduced into the bodies of susceptible animals, quickly invade the blood and multiply in it. In so doing they necessarily interfere with its physiological functions by appropriating for their own use material required for the nutrition of the tissues; and at the same time toxic substances are formed which play an important part in the production of the morbid phenomena, which in this class of diseases very commonly lead to a fatal result. The pathogenic bacteria which invade the blood may also, in certain cases, give rise to local necrosis and disturbance of function in various organs in a mechanical way by blocking up the capillaries.

The invasion of the blood which occurs in anthrax and in various forms of septicæmia in the lower animals, induced by subcutaneous inoculation with pure cultures of certain pathogenic bacteria, does not generally immediately follow the inoculation. Usually a considerable local development first occurs, which gives rise to more or less inflammation of the invaded tissues, and very commonly to an effusion of bloody serum in which the pathogenic microörganism is found in great numbers. Even in susceptible animals the blood seems to offer a certain resistance to invasion, which is overcome after a time by the vast number of the parasitic host located in the vicinity of the point of inoculation, aided probably by the toxic substances developed as a result of their vital activity.

The experiments of Cheyne (1886) seem to show that in the case of very pathogenic species, like the anthrax bacillus or Koch's bacillus of mouse septicæmia, a single bacillus introduced subcutaneously may produce a fatal result in the most susceptible animals, while greater numbers are required in those which are less susceptible. Thus a guinea-pig succumbed to general infection after being inoculated subcutaneously with anthrax blood diluted to such an extent that, by estimation, only one bacillus was present in the fluid injected; and a similar result in mice was obtained with Bacillus murisepticus. In the case of the microbe of fowl cholera (Bacillus septicæmiæ hæmorrhagicæ) Cheyne found that for rabbits the fatal dose is 300,000 or more, that from 10,000 to 300,000 cause a local abscess, and that less than 10,000 produce no appreciable effect. The common saprophyte Proteus vulgaris was found to be pathogenic for rabbits when injected into the dorsal muscles in sufficient numbers. But, according to the estimates made, 225,000,000 were required to cause death, while with doses of from 9,000,000 to 112,000,000 a local abscess was produced, and less than 9,000,000 gave an entirely negative result.

Secondary infections occurring in the course of specific infectious diseases are of common occurrence. Thus a pneumonia may be developed in the course of an attack of measles or of typhoid fever; or infection by the common pus cocci in the course of scarlet fever, typhoid fever, mumps, etc., may give rise to local abscesses, to endocarditis, etc. Again, *mixed infection* may be induced by injecting simultaneously into susceptible animals two species of pathogenic bacteria.

Bumm, Bockhart, and others have reported cases of mixed gonorrhœal infection in which the pyogenic micrococci gave rise to abscesses in the glands of Bartholin, to cystitis, parametritis, or to "gonorrhœal inflammation" of the knee joint. Babes gives numerous examples of mixed infection in scarlet fever and in other diseases of childhood. Anton and Fütterer have studied the question of secondary infection in typhoid fever. Karlinski has reported a case of secondary infection with anthrax in a case of typhoid fever, infection occurring by way of the intestine. Many other examples of secondary or mixed infection are recorded in the recent literature of bacteriology and clinical medicine, but enough has been said to call attention to the importance of the subject.

The researches of Römer, Kanthack (1892), and others show that the injection of the filtered products of certain bacteria (Bacillus pyocyaneus, Vibrio Metchnikovi, etc.) produces a decided leucocytosis in the animals experimented upon. And a similar result, probably from a like cause, has been shown by recent experiments to occur in pneumonia (Billings) and other infectious diseases.

Certain bacterial products have been shown by experiment to produce fever when injected into the circulation or beneath the skin of lower animals; others produce rapid respiration, dilatation of pupils, diarrhœa, and paralysis or convulsions (typhotoxin of Brieger, methyl-guanidin, etc.); the toxic effects of some are immediate and of others more or less remote (toxalbumin of diphtheria); others have a primary toxic effect which is followed after a time by toxic symptoms of a different order (Pneumobacillus liquefaciens bovis).

II.

CHANNELS OF INFECTION.

We have abundant evidence that susceptible animals may be infected by the injection of various pathogenic bacteria beneath the skin, and accidental infection *through an open wound or abrasion of the skin* is the common mode of infection in tetanus, erysipelas, hospital gangrene, and the "traumatic infectious diseases" generally. Other infectious diseases, like anthrax and glanders, are frequently transmitted in the same way. We have also satisfactory evidence that tuberculosis may be transmitted to man by the accidental inoculation of an open wound; and in view of the fact that susceptible animals are readily infected in this way, it would be strange if it were otherwise.

The question whether infection may occur through *the unbroken skin* has been studied by several bacteriologists and an affirmative result obtained. Thus Schimmelbusch produced pustules upon the thigh in two young persons suffering from pyæmia by rubbing upon the surface a pure culture of Staphylococcus pyogenes aureus which he had obtained from the pus of a furuncle. The same author also succeeded in infecting rabbits and guinea-pigs with anthrax, and rabbits with rabbit septicæmia, by rubbing pure cultures upon the uninjured skin. Similar results had previously been reported by Roth, who also showed that infection might occur through the uninjured mucous membrane of the nose. Machnoff also succeeded in infecting guinea-pigs with anthrax through the uninjured skin of the back, and, as a result of subsequent microscopical examination of stained sections, arrived at the conclusion that the principal channel through which infection was accomplished was the hair follicles. Braunschweig, in a series of experiments in which he introduced various pathogenic bacteria into the conjunctival sac of mice, rabbits, and guinea-pigs, obtained a negative result with the anthrax bacillus, the bacillus of mouse septicæmia, the bacillus of chicken cholera, and Micrococcus tetragenus; but the bacillus obtained by Ribbert from the intestinal diphtheria of rabbits gave a positive result in five mice, two guinea-pigs, and a rabbit.

Infection through the *mucous membrane of the intestine* no doubt occurs in certain diseases. This is believed to be a common mode of the infection of sheep and cattle with anthrax, and probably also in the infectious disease of swine known as hog cholera. The anthrax bacillus would be destroyed by the acid secretions of the stomach, but if spores are present in food ingested they will reach the intestine. The experiments of Korkunoff do not, however, support the view that infection is likely to occur in this way. In a series of experiments upon white mice fed with bread containing a quantity of anthrax spores the result was uniformly negative, but exceptionally infection occurred in rabbits. The same author obtained positive results in rabbits fed with food to which a pure culture of the bacillus of chicken cholera had been added.

Buchner, in experiments upon mice and guinea-pigs fed with material containing anthrax spores, obtained a positive result in four out of thirty-three animals. This is no doubt the usual mode of infection in typhoid fever in man.

Infection may also occur through the *mucous membrane of the respiratory organs.* This has been demonstrated by several bacteriologists, and especially by the experiments of Buchner, who mixed dried anthrax spores with lycopodium powder or pulverized charcoal, and caused mice and guinea-pigs to respire an atmosphere containing this powder in suspension. In a series of sixty-six experiments fifty animals died of anthrax, nine of pneumonia, and seven survived. That infection did not occur through the mucous membrane of the alimentary canal was proved by comparative experiments in which animals were fed with double the quantity of spores used in the inhalation experiments. Out of thirty-three animals fed in this way but four contracted anthrax. That infection occurred through the lungs was also demonstrated by the microscopical examination of sections and by culture experiments, which showed that the lungs were extensively invaded, while in many cases the spleen contained no bacilli. Positive results were also obtained with cultures of the anthrax bacillus not containing spores, which the animals were made to inhale in the form of spray. But in this case a considerable quantity was required, and a sero-fibrinous pneumonia was usually produced as well as general infection; the inhalation of small quantities gave no result. Positive results in rabbits were also obtained by causing them to inhale considerable quantities of a spray containing the bacillus of chicken cholera.

The fact that large quantities of a liquid culture of these virulent bacilli were required to infect very susceptible animals by way of the pulmonary mucous membrane, and that Buchner failed to cause the infection of these animals with small quantities of a pure culture inhaled in the form of spray, indicates that this is not a common mode of infection in the absence of spores. This view receives further support from the experiments of Hildebrandt, who made tracheal fistulæ in three rabbits, and, after the wound had entirely healed, injected into the trachea of each a pure culture of the anthrax bacillus, which was proved to be virulent by inoculation in mice or guinea-pigs. All of the animals remained in good health. On the other hand, three rabbits which received in the same way a pure culture of the bacillus of rabbit septicæmia died as a result of general infection.

That man may be infected with anthrax by way of the respiratory organs seems to be well established. In England the disease known as "wool-sorter's disease" results from infection in this way among workmen engaged in sorting wool, which is liable to contain the spores of the anthrax bacillus when obtained from the skin of an animal which has fallen a victim to this disease. That infection occurs through the lungs is shown by the fact that these organs are first involved, the disease being, in fact, a pulmonic anthrax.

While these experiments prove the possibility of infection through the respiratory mucous membrane, other experiments made by Hildebrandt show that under ordinary circumstances bacteria suspended in the air do not reach the trachea in rabbits, but are deposited upon the mucous membrane of the mouth, nares, and fauces. In healthy rabbits the tracheal mucus was, as a rule, found to be free from bacteria, while they were very numerous in mucus obtained from the mouth or nares. But when a rabbit was made to inhale for half an hour an atmosphere charged with the spores of Aspergillus fumigatus their presence in the lungs was demonstrated by cultivation, the animal being killed for the purpose half an hour after the inhalation experiment.

The rapidity with which infection may occur is shown by the experiments of Nissen, Pfuhl, and others. In mice inoculated with anthrax bacilli at the tip of the tail fatal anthrax has resulted, although the tail was amputated ten minutes after the inoculation. Schimmelbusch inoculated fresh wounds with anthrax cultures (in mice) and immediately after treated the wounds with strong antiseptic solutions, but the animals succumbed to infection. Cultures of the anthrax bacillus have been obtained from the liver, spleen, and kidneys half an hour after the infection of an open wound on the surface of the body (Schimmelbusch and Ricker). The experiments of Sherrington and others show that pathogenic bacteria may escape by way of the kidneys into the bladder, or through the liver into the gall bladder. But his experiments indicate that such escape does not occur through healthy organs. Non-pathogenic bacteria injected into the circulation were not found in the urine, and when a considerable quantity of a pathogenic species was injected into a vein there was no immediate appearance of bacteria in the urine, but they were found later, probably as a result of lesions in the secreting organ due to their local action or to that of their toxic products. In man the presence of pathogenic bacteria in the urine has been frequently verified, especially in typhoid fever, pneumonia, and streptococcus infection. When, as a result of the establishment of foci of infection in the liver, localized necrosis of tissue occurs, the pathogenic bacteria to which the infection is due escape with the bile and enter the intestine. It is probable that escape through the walls of the intestine does not occur unless there is a local lesion of some kind, as in typhoid fever.

The presence of tubercle bacilli in the milk of cows has been repeatedly demonstrated, and in a certain proportion of the cases they have been found in the milk of cows whose udders gave no evidence of being the seat of a tubercular process. Usually, however, when tubercle bacilli are found in the milk the cow's udder is already involved in the disease. The milk of women with puerperal fever has been found to contain streptococci; and in mastitis from a localized infection by pyogenic cocci these are found in the milk. It must be remembered, however, that both Staphylococcus albus and aureus have been found in the milk of healthy women. The micrococcus of pneumonia has been found in the milk of women suffering from croupous pneumonia (Foà, and Bordoni-Uffreduzzi). Various observers (Brunner, Tizzoni, von Eiselsberg) have reported the presence of pus cocci in the sweat of patients suffering from septicæmia, and the experiments of Brunner indicate that they may have escaped through the sweat glands. This, however, does not appear to be definitely established.

III.

SUSCEPTIBILITY AND IMMUNITY.

No questions in general biology are more interesting, or more important from a practical point of view, than those which relate to the susceptibility of certain animals to the pathogenic action of certain species of bacteria, and the immunity, natural or acquired, from such pathogenic action which is possessed by other animals. It has long been known that certain infectious diseases, now demonstrated to be of bacterial origin, prevail only or principally among animals of a single species. Thus typhoid fever, cholera, and relapsing fever are diseases of man, and the lower animals do not suffer from them when they are prevailing as an epidemic. On the other hand, man has a natural immunity from many of the infectious diseases of the lower animals, and diseases of this class which prevail among animals are frequently limited to a single species. Again, several species, including man, may be susceptible to a disease, while other animals have a natural immunity from it. Thus tuberculosis is common to man, to cattle, to apes, and to the small herbivorous animals, while the carnivora are, as a rule, immune; anthrax may be communicated by inoculation to man, to cattle, to sheep, to guinea-pigs, rabbits, and mice, but the rat, the dog, carnivorous animals, and birds are generally immune; glanders, which is essentially a disease of the equine genus, may be communicated to man, to the guinea-pig, and to field mice, while house mice, rabbits, cattle, and swine are to a great extent immune.

In addition to this general race immunity or susceptibility we have individual differences in susceptibility or resistance to the action of pathogenic bacteria, which may be either natural or acquired. As a rule, *young animals are more susceptible than older ones.* Thus in man the young are especially susceptible to scarlet fever, whooping cough, and other "children's diseases," and after forty years of age the susceptibility to tubercular infection is very much diminished. Among the lower animals it is a matter of common laboratory experience that the very young of a susceptible species may be infected when inoculated with an "attenuated culture" which older animals of the same species are able to resist.

Considerable differences as to susceptibility may also exist among adults of the same species. In man these differences in individual susceptibility to infectious diseases are frequently manifested. Of a number of persons exposed to infection in the same way, some may escape entirely while others have attacks differing in severity and duration. In our experiments upon the lower animals we constantly meet with similar results, some individuals proving to be exceptionally resistant. Exceptional susceptibility or immunity may be to some extent a family characteristic or one of race. Thus the negro race is decidedly less subject to yellow fever than the white race, and this disease is more fatal among the fair-skinned races of the north of Europe than among the Latin races living in tropical or subtropical regions. On the other hand, small-pox appears to be exceptionally fatal among negroes and dark-skinned races generally.

A very remarkable instance of race immunity is that of Algerian sheep against anthrax, a disease which is very fatal to other sheep.

In the instances mentioned *race immunity* is probably an acquired tolerance due to natural selection and inheritance. If, for example, a susceptible population is exposed to the ravages of small-pox, the least susceptible individuals will survive and may be the parents of children who will be likely to inherit the special bodily characters upon which this comparative immunity depends. The tendency of continuous or repeated exposure to the same pathogenic agent will evidently be to establish a race tolerance ; and there is reason to believe that such has been the effect in the case of some of the more common infectious diseases of man, which have been noticed to prevail with especial severity when first introduced among a virgin population, as in the islands of the Pacific, etc.

In the same way we may explain the immunity which carnivorous animals have for anthrax and various forms of septicæmia to which the herbivora are very susceptible when the pathogenic germ is introduced into their bodies by inoculation. From time immemorial the carnivora have been in the habit of fighting over the dead bodies of herbivorous animals, some of which may have fallen a prey to these infectious germ diseases, and in their fighting they receive wounds, inoculated with the infectious material from these bodies, which would be fatal to a susceptible animal. If at any time in the past a similar susceptibility existed among the carnivora, with individual differences as to resisting power, it is evident that there would be a constant tendency for the most susceptible individuals to perish and for the least susceptible to survive.

But if we admit this to be a probable explanation of the immunity of carnivorous animals from septic infection, we have not yet explained the precise reason for the immunity enjoyed by the selected individuals and their progeny. The essential difference between a susceptible and immune animal depends upon the fact that in one the pathogenic germ, when introduced by accident or experimental inoculation, multiplies and invades the tissues or the blood, where, by reason of its nutritive requirements and toxic products, it produces changes in the tissues and fluids of the body inconsistent with the vital requirements of the infected animal; while in the immune animal multiplication does not occur or is restricted to a local invasion of limited extent, and in which after a time the resources of nature suffice to destroy the parasitic invader.

Now the question is, upon what does this essential difference depend? Evidently upon conditions favorable or unfavorable to the development of the pathogenic germ; or upon its destruction by some active agent present in the tissues or fluids of the body of the immune animal; or upon a neutralization of its toxic products by some substance present in the body of the animal which survives infection.

What, then, are the unfavorable conditions which may be supposed to prevent development in immune animals? In the first place, the temperature of the body may not be favorable. Certain pathogenic bacteria are only able to develop within very narrow temperature limits, and, if all other conditions were favorable, could not be expected to multiply in the bodies of cold-blooded animals. Or the temperature of warm-blooded animals, and especially of fowls, may be above the point favorable for their development. This is the explanation offered by Pasteur of the immunity of fowls, which are usually refractory against anthrax; and in support of this view he showed by experiment that when chickens are refrigerated after inoculation, by being partly immersed in cold water, they are liable to become infected and to perish. But, as pointed out by Koch, the sparrow, which has a temperature as high as that of the chicken, may contract anthrax without being refrigerated. We must not, therefore, too hastily conclude that the success in Pasteur's experiment depended alone upon a reduction of the body heat. Gibier has shown that the anthrax bacillus may multiply in the bodies of frogs or fish, if these are kept in water having a temperature of 35° C. But the anthrax bacillus grows within comparatively wide temperature limits, while other pathogenic bacteria are known to have a more restricted temperature range and would be more decidedly influenced by this factor—*e.g.*, the tubercle bacillus.

The composition of the body fluids, and especially their reaction, is probably a determining factor in some instances. Thus Behring has ascribed the failure of the anthrax bacillus to develop in the white rat, which possesses a remarkable immunity against anthrax, to the highly alkaline reaction of the blood and tissue juices of this animal. Behring claims to have obtained experimental proof of the truth of this explanation by feeding white rats on an exclusively vegetable diet or by adding acid phosphate of lime to their food, by which means this excessive alkalinity of the blood is diminished. Rats so treated are said to lose their natural immunity, and to die as a result of inoculation with virulent cultures of the anthrax bacillus.

The experiments of Nuttall, Behring, Buchner, and others have established the fact that *recently drawn blood of various animals possesses decided germicidal power*, and Buchner has shown that this property belongs to the fluid part of the blood and not to its cellular elements. It has also been shown that aqueous humor, the fluid of ascites, and lymph from the dorsal lymph sac of a frog possess the same power. This power to kill bacteria is destroyed by heat, and is lost when the blood has been kept for a considerable time, but it is not neutralized by freezing. Further, this power to destroy bacteria differs greatly for different species, being very decided in the case of certain pathogenic bacteria, less so for others, and absent in the case of certain common saprophytes. Behring has also shown that the blood of different animals differs considerably in this regard, and that the blood of the rat and of the frog, which animals have a natural immunity against anthrax, is especially fatal to the anthrax bacillus. The experiments made show that this germicidal power is very prompt in its action, but that it is limited as to the number of bacteria which can be destroyed by a given quantity of blood serum. When the number is excessive, development occurs after an interval during which a limited destruction has taken place. It would appear that the element in the blood to which this germicidal action is due is neutralized in exercising this power ; and as, independently of this, blood serum is an excellent culture medium for bacteria, an abundant development takes place when the destruction has been incomplete.

Buchner (1889) first proved by experiment that the germicidal power of the blood of dogs and rabbits does not depend upon the presence of the cellular elements, but is present in clear serum which has been allowed to separate from the clot in a cool place. Exposure for an hour to a temperature of 55° C. destroys the germicidal action of serum as well as of blood.

The researches of Buchner, of Hankin, and others, show that this germicidal power of fresh blood serum depends upon the presence of proteids, to which the first-named bacteriologist has given the name of "alexins." Hankin, in his paper upon the origin of these "defensive proteids" in the animal body (1892), arrives at the conclusion that while they are present in the cell-free serum they are the product of certain leucocytes—Ehrlich's eosinophile cells. He believes that the eosinophile granules become dissolved in the serum and constitute the germicidal proteid which is shown to be present by experiments upon bacteria. According to Hankin the separation of these granules can be witnessed under the microscope. They first accumulate upon one side of the cell and then gradually disappear, and as this occurs a considerable increase in the bactericidal power of the serum can be demonstrated. The germicidal power of the blood serum is also said to be increased when the number of leucocytes is considerably augmented, as occurs when a sterilized culture of Vibrio Metschnikovi is injected subcutaneously. Also by treatment which favors a separation of the alexin from the leucocytes, *i.e.*, a solution of the eosinophile granules. This may be accomplished by the injection of an extract of the thymus gland of the calf, or by simply allowing the drawn blood to stand for several hours at a temperature of 38° to 40° C.

Buchner's latest communication upon the subject shows that he also attributes the origin of the germicidal proteid in fresh blood serum to the leucocytes. In his paper on "Immunity," read at the Eighth International Congress on Hygiene and Demography (Budapest, 1894), he calls attention in the first place to the fact that a clearly marked distinction must be made between natural immunity and acquired immunity, inasmuch as the "alexins" and "antitoxins" have very different properties. The first-mentioned proteids are destroyed by a comparatively low temperature (55° to 60° C.), while the antitoxins resist a considerably higher temperature, and, unlike the alexins, have no bactericidal or globulicidal action. A very remarkable fact developed in Buchner's experiments is that the blood serum from the dog and from the rabbit, when mixed, neutralize each other so far as their germicidal power is concerned.

By injecting sterilized emulsions of wheat-flour paste in the pleural cavity of rabbits and dogs Buchner succeeded in obtaining an exudate which had more decided germicidal power than the blood or serum of the same animal. This was evidently due to the large number of leucocytes present, but not to their phagocytic action, as was shown by experiment. By freezing the exudate the leucocytes were killed, but the germicidal action of the fluid was rather increased than diminished by freezing. While freezing had no effect upon the germicidal action of the pleural exudate, this was always neutralized by exposure to a temperature of 55° C.

Emmerich, Tsuboi, Steinmetz, and Löw (1892), as a result of extended experiments, arrived at the conclusion that the germicidal action of blood serum "depends upon a specific property of the alkali serumalbumin, and that it is a purely chemical process." They state that when the germicidal power is neutralized by heat it may be restored by the addition of an alkali. Buchner repeated the experiments of Emmerich and his associates and obtained similar results, but interprets them differently. According to him the serum does not regain its germicidal power, but after the addition of an alkali and subsequent dialyzing the nutritive value of the serum is so diminished that the bacteria do not develop in it.

Pane (1892) has made experiments which give additional weight to the assumption that the alkalinity of the blood is an important factor in accounting for immunity. He states that carbonate of soda, dissolved in water, in the proportion of 1:3,000, has a decided germicidal action upon the anthrax bacillus, equal to that of the blood serum of the rabbit. And that when rabbit serum is completely neutralized it no longer has any injurious action on anthrax bacilli.

Zagari and Innocente (1892) also arrived at the conclusion that the diminished resistance to anthrax infection resulting from curare poisoning in frogs, and from chloral or alcohol in dogs (Platania), in fowls as a result of starvation (Canalis and Morpurgo), in white mice as a result of fatigue (Charin and Roger), is, in fact, due to diminished alkalinity of the blood, which they found to correspond with the increased susceptibility resulting from the causes mentioned.

Buchner (1892) states that several of the ammonium salts, and especially ammonium sulphate, cause an increase in the germicidal action of blood serum, and also increase its resistance to the neutralizing effects of heat. The experiments of Pansini and Calabrese (1894) show, on the contrary, that the addition of uric acid to blood serum diminishes its bactericidal activity, as does also the presence of glucose. That certain infectious diseases are especially virulent in persons suffering from diabetes is a frequently repeated clinical observation.

Van Fodor has shown by experiment that the injection of an alkali into the circulation of a rabbit increases its resistance to anthrax infection and the germicidal activity of its blood serum. The same bacteriologist has found that when a rabbit is infected with anthrax, the alkalinity of its blood is notably increased during the first twenty-four hours, when we may suppose that the powers of nature are brought to bear to resist the invading parasite, and that after this time it rapidly diminishes. Ten hours after infection (by subcutaneous inoculation?) the alkalinity of the blood had increased 21.5 per cent. Shortly before the death of the animal a diminution of 26.3 per cent was noted. This diminution was observed in thirty-four out of thirty-nine animals experimented upon, and these animals succumbed to the anthrax infection in a shorter time than did the other five in which there was no such diminution.

It seems probable that the germicidal property of freshly drawn blood serum is not due to its alkalinity, *per se*, but to the fact that the germicidal constituent is only soluble in an alkaline fluid. The researches of Vaughn, McClintock, and Novy indicate that this germicidal constituent is a nuclein. Dr. Vaughn, in his last published paper upon "Nucleins and Nuclein Therapy," says: "Kossel, of Berlin, has confirmed our statements concerning the germicidal action of the nucleins. Dr. McClintock and I have also demonstrated that the germicidal constituent of blood serum is a nuclein. This nuclein is undoubtedly furnished by the polynuclear white corpuscles." Denys has (1894) reported the results of experiments made in his laboratory by Van der Velde, which give support to the conclusion reached by Vaughn. In these experiments a sterilized culture of staphylococci was injected into the pleural cavity of rabbits in order to obtain an exudate. At intervals of two hours this exudate was obtained by killing one of the animals in the series experimented upon, and at the same time blood from the animal was secured. Both the exudate and the blood were placed in a centrifugal machine, in order to obtain a serum free from corpuscular elements. The germicidal activity of the serum was then tested. The general result of the experiments was to show that the longer the interval after the injection into the pleural cavity the more potent the germicidal activity of the exudate became, and that there was no corresponding increase in the activity of the blood serum obtained from the circulation. At the end of ten or twelve hours, the serum from the exudate killed all of the staphylococci in a bouillon culture twenty times as great in quantity as the germicidal serum used in the experiment. The absence of any increase in germicidal power in the blood serum taken from the general circulation shows that the notable increase manifested by the exudate was due to local causes; and as a matter of fact it corresponded with an increase in the number of leucocytes as found in the pleural exudate.

Thus it will be seen that the independent researches of Hankin, of Buchner, of Vaughn, and of other competent bacteriologists, have led them to the same ultimate result so far as the origin of the germicidal constituent of the blood is concerned, and that the leucocytes appear to play an important *rôle* in the protection of the animal body from invasion by bacteria (natural immunity).

It has been shown by several investigators that the number of leucocytes increases in certain infectious diseases, and this increase, together with an increased alkalinity of the blood, which has heretofore been referred to, appears to be a provision of nature for overcoming the infection which has already occurred.

It has been demonstrated by experiment that naturally immune animals may be infected by the addition of certain substances to cultures of pathogenic bacteria. Thus Arloing was able to induce symptomatic anthrax in animals naturally immune for this disease by mixing with his cultures various chemical substances, such as carbolic acid, pyrogallic acid, and especially lactic acid (twenty per cent). Leo has shown that white mice, which are not subject to the pathogenic action of the glanders bacillus, may be rendered susceptible by feeding them for some time upon phloridzin, which gives rise to an artificial diabetes, and causes the tissues to become impregnated with sugar.

Bouchard has found that very small doses of a pure culture of Bacillus pyocyaneus are fatal to rabbits when at the same time a considerable quantity of a filtered culture of the same bacillus is injected into a vein. The animal could have withstood the filtered culture alone, or the bacillus injected beneath its skin; but its resisting power—natural immunity—is overcome by the combined action of the living bacilli and the toxic substances contained in the filtered culture. The same result may be obtained by injecting sterilized cultures of a different microörganism. Thus Roger has shown that the rabbit, which has a natural immunity against symptomatic anthrax, succumbs to infection when inoculated with a culture of the bacillus of this disease, if at the same time it receives an injection of a sterilized or non-sterilized culture of Bacillus prodigiosus. Monti has succeeded in killing animals with old and attenuated cultures of Streptococcus pyogenes, or of Staphylococcus pyogenes aureus, by injecting at the same time a culture of Proteus vulgaris. In a similar way, it seems probable, the normal resistance of man to infection by certain pathogenic bacteria may be overcome. Thus when water contaminated by the presence of the typhoid bacillus is used for drinking by the residents of a certain town or district, not all of those who in this way are exposed to infection contract typhoid fever; and among those who do, there is good reason to believe that, in certain cases at least, the result depends upon an additional factor of the kind suggested by the above-mentioned experiments—*e.g.*, the consumption of food containing putrefactive products, or the respiration of an atmosphere containing volatile products of putrefaction.

The natural immunity of healthy animals may also be neutralized by other agencies which have a depressing effect upon the vital resisting power. Thus Nocard and Roux found by experiment that an attenuated culture of the anthrax bacillus, which was not fatal to guinea-pigs, killed these animals when injected into the muscles of the thigh after they had been bruised by mechanical violence. Abarrin and Roger found that white rats, which are not susceptible to anthrax, became infected and frequently died if they were exhausted, previous to inoculation, by being compelled to turn a revolving wheel for a considerable time. Pasteur found that fowls, which have a natural immunity against anthrax, become infected and perish if they are subjected to artificial refrigeration after inoculation. This has been confirmed by the more recent experiments of Wagner (1891). According to Canalis and Morpurgo, pigeons which are enfeebled by inanition eaily contract anthrax as a result of inoculation. Arloing states that sheep which have been freely bled contract anthrax more easily than others; and Serafini found that when dogs were freely bled the bacillus of Friedländer, injected into the trachea or the pleural cavity, entered, and apparently multiplied to some extent in the blood, whereas without such previous bleeding they were not to be found in the circulating fluid. Certain anæsthetic agents have been shown also to produce a similar result. Platania communicated anthrax to immune animals—dogs, frogs, pigeons—by bringing them under the influence of curare, chloral, or alcohol; and Wagner obtained similar results in his experiments upon pigeons to which he had administered chloral. In man, clinical experience shows that those who are addicted to the excessive use of alcohol are especially liable to contract certain infectious diseases —pneumonia, erysipelas, yellow fever, etc.

The micrococcus of pneumonia is habitually present in the salivary secretions of many healthy individuals, and it is evident that an attack of pneumonia does not depend alone upon the presence of this micrococcus, which has, nevertheless, been conclusively shown to be the usual infectious agent in cases of croupous pneumonia. No doubt the introduction of the pathogenic micrococcus to the vulnerable point—the lungs—is an essential factor in the development of a case of pneumonia, but there is reason to believe that there are other factors equally essential. Thus it is well known that an attack of pneumonia often results from exposure to cold, which may act as an exciting cause; and, also, that a recent attack of an acute febrile disease—especially measles—constitutes a predisposing cause. It is generally recognized that malnutrition, want of exercise, insanitary surroundings, and continued respiration of an atmosphere loaded with dust, as in cotton mills, or a recent attack of pneumonia, constitute predisposing causes to tubercular infection by way of the lungs.

While natural immunity may be overcome by the various depressing agencies referred to, it is also true that it has only a relative value in the absence of these predisposing causes, and may be overcome by unusual virulence of the pathogenic infectious agent, or by the introduction into the body of an excessive amount of a pure culture of the same.

The pathogenic potency of known disease germs varies as widely as does the susceptibility of individuals to their specific action. In general it may be said that the more recently the germ comes from a developed case of the disease to which it gives rise the more virulent it is, and the longer it has been cultivated outside of the animal body the more attenuated is its pathogenic power. Thus when the discharges of a typhoid fever patient find their way directly to a water-supply of limited amount a large proportion of those who drink the water are likely to be attacked; but when a considerable interval of time has elapsed since the contamination occurred, although the germs may still be present, the liability to attack is much less on account of diminished pathogenic virulence.

The development of an attack also depends, to some extent, upon the number of germs introduced into a susceptible individual at one time. The resources of nature may be sufficient to dispose of a few bacilli, while a large number may overwhelm the resisting power of the individual.

The experiments of Cheyne (1886) show that in the case of very pathogenic species a single bacillus, or at least a very small number, introduced beneath the skin, may produce fatal infection in a very susceptible animal, while greater numbers are required in those less susceptible. Thus a guinea-pig succumbed to general infection after being inoculated subcutaneously with anthrax blood diluted to such an extent that, by estimation, only one bacillus was present in the fluid injected; and a similar result was obtained in mice with Bacillus murisepticus. In the case of the microbe of fowl cholera (Bacillus septicæmia hemorrhagicæ), Cheyne found that for rabbits the fatal dose was 300,000 or more, that from 100,000 to 30,000 cause a local abscess, and that less than 10,000 produce no appreciable effect. The common saprophyte, Proteus vulgaris, was found to be pathogenic for rabbits when injected into the dorsal muscles in sufficient numbers. But, according to the estimates made, 225,000,000 were required to cause death, while doses of from 9,000,000 to 112,000,000 produced a local abscess, and less than 9,000,000 gave an entirely negative result.

ACQUIRED IMMUNITY.

It has long been known that, in a considerable number of infectious diseases, a single attack, however mild, affords protection against subsequent attacks of the same disease; that in some cases this protection appears to be permanent, lasting during the life of the individual; that in others it is more or less temporary, as shown by the occurrence of a subsequent attack.

The protection afforded by a single attack not only differs in different diseases, but in the same disease varies greatly in different individuals. Thus certain individuals have been known to suffer several attacks of small-pox or of scarlet fever, although, as a rule, a single attack is protective. Exceptional susceptibility or insusceptibility may be not only an individual but a family characteristic, or it may belong to a particular race.

In those diseases in which second attacks are not infrequent, as, for example, in pneumonia, in influenza, or in Asiatic cholera, it is difficult to judge from clinical experience whether a first attack exerts any protective influence. But from experiments upon the lower animals we are led to believe that a certain degree of immunity, lasting for a longer or shorter time, is afforded by an attack of pneumonia or of cholera, and probably of all infectious diseases due to bacterial parasites. In the malarial fevers, which are due to a parasite of a different class, one attack affords no protection, but rather predisposes to a subsequent attack.

In those diseases in which a single attack is generally recognized as being protective, exceptional cases occur in which subsequent attacks are developed as a result of unusual susceptibility or exposure under circumstances especially favorable to infection. Maiselis (1894) has gone through the literature accessible to him for the purpose of determining the frequency with which second attacks occur in the various diseases below mentioned. The result is as follows:

	Second Attacks.	Third Attacks.	Fourth Attacks.	Total.
Small-pox	505	9	0	514
Scarlet fever	29	4	0	33
Measles	36	1	0	37
Typhoid fever	202	5	1	208
Cholera	29	3	2	34

These figures support the view generally entertained by physicians that second attacks of scarlet fever and of measles are comparatively rare, while second attacks of small-pox are not infrequently observed. Considering the very large number of cases of typhoid fever which occur annually in all parts of Europe and America, the number of second attacks collected does not bear a very large proportion to the total number taken sick, although the recorded cases, of course, fall far short of the total number of second attacks of this and the other diseases mentioned.

The second attacks of cholera recorded are not numerous, and, no doubt, a carefullly conducted investigation made in the areas of endemic prevalence of this disease would show that second attacks are more common than is indicated by these figures.

That immunity may result from a comparatively mild attack as well as from a severe one is a matter of common observation in the case of small-pox, scarlet fever, yellow fever, etc.; and since the discovery of Jenner we have in vaccination a simple method of producing immunity in the first-mentioned disease. The acquired immunity resulting from vaccination is not, however, as complete or as permanent as that which results from an attack of the disease.

These general facts relating to acquired immunity from infectious diseases constituted the principal portion of our knowledge with reference to this important matter up to the time that Pasteur (1880) demonstrated that in the disease of fowls known as chicken cholera, which he had proved to be due to a specific microörganism, a mild attack followed by immunity may be induced by inoculation with an "attenuated virus"—*i.e.*, by inoculation with a culture of the pathogenic microörganism the virulence of which had been so modified that it gave rise to a comparatively mild attack of the disease in question. Pasteur's original method of obtaining an attenuated virus consisted in exposing his cultures for a considerable time to the action of atmospheric oxygen. It has since been ascertained that the same result is obtained with greater certainty by exposing cultures for a given time to a temperature slightly below that which would destroy the vitality of the pathogenic microörganism, and also by exposure to the action of certain chemical agents.

Pasteur at once comprehended the importance of his discovery, and inferred that what was true of one infectious germ disease was likely to be true of others. Subsequent researches, by this savant and by other bacteriologists, have justified this anticipation, and the demonstration has already been made for a considerable number of similar diseases—anthrax, symptomatic anthrax, rouget.

A virus which has been attenuated artificially—by heat, for example—may be cultivated through successive generations without regaining its original virulence. As this virulence depends, to a considerable extent at least, upon the formation of toxic products during the development of the pathogenic microörganism, we naturally infer that diminished virulence is due to a diminished production of these toxic substances.

There is reason to believe that a natural attenuation of virulence may occur in pathogenic bacteria which are able to lead a saprophytic existence during their multiplication external to the bodies of living animals, and the comparatively mild character of some epidemics is probably due to this fact.

Again, cultivation within the body of a living animal may, in certain cases, cause a diminution in the virulence of a pathogenic microörganism. Thus Pasteur and Thuiller have shown that the *microbe* of rouget when inoculated into a rabbit kills the animal, but that its pathogenic virulence is nevertheless so modified that a culture made from the blood of a rabbit killed by it is a suitable "vaccine" for the pig.

On the other hand, we have experimental evidence that the virulence of attenuated cultures may be reëstablished by passing them through the bodies of susceptible animals. Thus a culture of the bacillus of rouget, attenuated by having been passed through the body of a rabbit, is restored to its original virulence by passing it through the bodies of pigeons. And a culture of the anthrax bacillus which will not kill an adult guinea-pig may be fatal to a very young animal of the same species or to a mouse, and the bacillus cultivated from the blood of such an animal will be found to have greatly increased virulence.

In Pasteur's inoculations against anthrax "attenuated" cultures are employed which contain the living pathogenic germ as well as the toxic products developed during its growth. Usually two inoculations are made with cultures of different degrees of attenuation—that is to say, with cultures in which the toxic products are formed in less amount than in virus of full power. The most attenuated virus is first injected, and after some time the second vaccine, which if injected first might have caused a considerable mortality. The animal is thus protected from the pathogenic action of the most virulent cultures.

Now, it has been shown by recent experiments that a similar immunity may result from the injection into a susceptible animal of the toxic products contained in a virulent culture, independently of the living bacteria to which they owe their origin. Chauveau, in 1880, ascertained that if pregnant ewes are protected against anthrax by inoculation with an attenuated virus, their lambs, when born, also give evidence of having acquired an immunity from the disease. As the investigations of Davaine seemed to show that the anthrax bacillus cannot pass through the placenta from the mother to the fœtus, the inference seemed justified that the acquired immunity of the latter was due to some soluble substance which could pass the placental barrier. More recent researches by Strauss and Chamberlain, Malvoz and Jacquet, and others, show that the placenta is not such an impassable barrier for bacteria as was generally believed at the time of Chauveau's experiments, so that these cannot be accepted as establishing the inference referred to. But, as stated, we have more recent experimental evidence which shows that immunity may result from the introduction into the bodies of susceptible animals of the toxic substances produced by certain pathogenic bacteria. The first satisfactory experimental evidence of this important fact was obtained by Salmon and Smith in 1886, who succeeded in making pigeons immune from the pathogenic effects of cultures of the bacillus of hog cholera by inoculating them with sterilized cultures of this bacillus. In 1888 Roux reported similar results obtained by injecting into susceptible animals sterilized cultures of the anthrax bacillus. Behring and Kitasato, in 1890, reported their success in establishing immunity against virulent cultures of the bacillus of tetanus and the diphtheria bacillus by inoculating susceptible animals with filtered, germ-free cultures of these pathogenic bacteria.

In 1892 Behring, Kitasato, and Wassermann published the results of interesting experiments with a bouillon made from the thymus gland of the calf. They found that the tetanus bacillus cultivated in this bouillon did not form spores and had comparatively little virulence. Mice or rabbits inoculated with it in small doses—0.001 to 0.2 cubic centimetre for a mouse—proved to be subsequently immune. And the blood serum of an immune rabbit injected into the peritoneal cavity of a mouse—0.1 to 0.5 cubic centimetre—was found to give it immunity from the pathogenic action of a virulent culture of the tetanus bacillus. Similar results were obtained with several other pathogenic bacteria cultivated in the thymus bouillon—spirillum of cholera, bacillus of diphtheria, typhoid bacillus. We give here the directions for preparing the thymus bouillon as used by the authors named:

> Two or three thymus glands are chopped into small pieces immediately after they are taken from the animal. An equal part of distilled water is added to the mass and stirred for some time ; it is then placed in an ice chest for twelve hours. The juices are now expressed through gauze by means of a flesh press. A clouded, slimy fluid is obtained, which constitutes a stock solution. This is diluted with water, and a certain quantity of carbonate of soda is added to the solution before sterilization. By this means coagulation and precipitation of the active substance from the thymus gland are avoided. The exact amount of water and of sodium carbonate required to prevent precipitation must be determined by experiment, as it differs for different glands. Usually an equal portion of water and sufficient soda solution to turn litmus paper feebly blue will give the desired result. The liquid is now heated in a large flask, which is left for fifteen minutes in the steam sterilizer. The liquid is allowed to cool and then filtered through fine linen to remove any suspended coagula ; the filtrate has a milky opalescence. It is now placed in test tubes and again sterilized. The active principle is precipitated by the addition of a few drops of acetic acid.

In Pasteur's inoculations against hydrophobia, made subsequently to infection by the bite of a rabid animal, an attenuated virus is introduced upon the surface of the brain, and immunity is established during the interval—so-called period of incubation—which usually occurs between the date of infection and the development of the disease. That the immunity in this case also depends upon the introduction of a chemical substance present in the desiccated spinal cord of rabbits which have succumbed to rabies, which is used in these inoculations, is extremely probable. But, as the germ of rabies has not been isolated or cultivated artificially, this has not yet been demonstrated. Wooldridge claims to have made susceptible animals immune against anthrax by inoculating them with an aqueous extract of the testicle or of the thymus gland of healthy animals.

We may mention also the interesting results obtained by Emmerich, Freudenreich, and others, who have shown that an anthrax infection in a susceptible animal inoculated with a virulent culture may be made to take a modified and non-fatal course by the simultaneous or subsequent inoculation of certain other non-pathogenic bacteria—streptococcus of erysipelas, Bacillus pyocyaneus.

In a series of experiments made by the writer some years ago evidence was obtained that, under certain circumstances, immunity from the effects of one pathogenic bacillus may be obtained by the previous injection of a pure culture of a different species. In the experiments referred to injections into the cavity of the abdomen of a culture of Bacillus pyocyaneus protected rabbits from the lethal effects of Bacillus cuniculicida Havaniensis, when subsequently injected into the cavity of the abdomen in such amount (one cubic centimetre of a bouillon culture) as invariably proved fatal in rabbits not protected by such injections.

Before considering the theories which have been offered in explanation of acquired immunity it is desirable to call attention to certain observations which have been made during the past few years relating to "chemiotaxis."

The term *chemiotaxis* was first used by Pfeffer to designate the property, observed by himself and others, which certain living cells exhibit with reference to non-living organic material, and by virtue of which they approach or recede from certain substances. The chemiotaxis is said to be positive when the living cell approaches, and negative when it recedes from, a chemical substance. As examples of this we may mention the approach of motile bacteria to nutrient material or to the surface of a liquid medium where they find the oxygen required for their vital activities ; and of leucocytes to certain substances when these are introduced beneath the skin of warm- or cold-blooded animals. This subject has recently received much attention and has been studied especially by Ali-Cohen, Massart and Bordet, Gabritchevski, and others.

According to Gabritchevski, the following substances have a negative chemiotaxis for the leucocytes: Sodium chloride in ten-per-cent solution, alcohol in ten-per-cent solution, quinine, lactic acid, glycerin, chloroform, bile. On the other hand, a positive chemiotaxis is excited by sterilized or non-sterilized cultures of various bacteria. This is shown by the fact that when a small capillary tube, closed at one end, which contains the substance to be tested, is introduced beneath the skin of an animal, the leucocytes are repelled from the tube by certain substances, while those which incite positive chemiotaxis cause them to enter the tube in great numbers. The experiments of Buchner seem to show that the positive chemiotaxis induced by sterilized cultures of bacteria introduced beneath the skin of an animal, is due to the proteid contents of the cells rather than to the chemical products elaborated as a result of their vital activity. But that such chemical products may, in some instances at least, produce a positive chemiotaxis independently of the bacteria is shown by the experiments of Gabritchevski with filtered cultures of Bacillus pyocyaneus—confirmed by Massart and Bordet.

An important observation made by Bouchard, and confirmed by Massart and Bordet, is the following: When a tube containing a culture of Bacillus pyocyaneus is introduced beneath the skin of a rabbit it is found, at the end of a few hours, to contain a great number of leucocytes. But if immediately after its introduction ten cubic centimetres of a sterilized culture of the same bacillus are injected into the circulation through a vein, very few leucocytes enter the tube introduced beneath the skin—that is, the chemiotaxis of the leucocytes for the bacilli contained in the tube has been neutralized by injecting a considerable quantity of the soluble products of the same bacillus into the circulation.

Buchner, having shown that the bacterial cells contain a proteid substance which attracts the leucocytes, experimented with various other proteids and found that gluten, casein from wheat, and legumin from peas had a similar effect. Starch has no effect, but a mass of flour, made from wheat or from peas, introduced beneath the skin of a rabbit or of a guinea-pig, with antiseptic precautions, in the course of a day or two is enveloped and penetrated by immense numbers of leucocytes. If, instead of introducing these substances which induce positive chemiotaxis beneath the skin, they are injected into the circulation, Buchner has shown that a great increase in the number of leucocytes occurs.

THEORIES OF IMMUNITY.

Exhaustion Theory.—For a time Pasteur supported the view that during an attack of an infectious disease the pathogenic microörganism, in its multiplication in the body of a susceptible animal, exhausts the supply of some substance necessary for its development, that this substance is not subsequently reproduced, and that consequently the same pathogenic germ cannot again multiply in the body of the protected animal. This view is sustained in a memoir published in the *Comptes Rendus* of the French Academy in 1880, in which Pasteur says :

> "It is the life of a parasite in the interior of the body which produces the malady commonly called '*choléra des poules*,' and which causes death. From the moment when this culture (*i.e.*, the multiplication of the parasite) is no longer possible in the fowl the sickness cannot appear. The fowls are then in the constitutional state of fowls not subject to be attacked by the disease. These last are as if vaccinated from birth for this malady, because the fœtal evolution has not introduced into their bodies the material necessary to support the life of the microbe, or these nutritive materials have disappeared at an early age.
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> "Certainly one should not be surprised that there may be constitutions sometimes susceptible and sometimes rebellious to inoculation—that is to say, to the cultivation of a certain virus—when, as I have announced in my first note, one sees a preparation of beer yeast made, exactly like one from the muscles of fowls (*bouillon*), to show itself absolutely unsuited for the cultivation of the parasite of fowl cholera, while it is admirably adapted to the cultivation of a multitude of microscopic species, notably to the *bactéride charbonneuse* (Bacillus anthracis).
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> "The explanation to which these facts conduct us, as well of the constitutional resistance of some individuals as of the immunity produced by protective inoculations, is only natural when we consider that every culture, in general, modifies the medium in which it is effected—a modification of the soil when it relates to ordinary plants; a modification of plants and animals when it relates to their parasites ; a modification of our culture liquids when it relates to *mucédines*, *vibrioniens*, or ferments.
>
> "These modifications are manifested and characterized by the circumstance that new cultivations of the same species in these media become promptly difficult or impossible. If we sow *chicken bouillon* with the microbe of fowl cholera, and, after three or four days, filter the liquid in order to remove all trace of the microbe, and subsequently sow anew in the filtered liquid this parasite, it will be found quite powerless to resume the most feeble development. The liquid, which is perfectly limpid after being filtered, retains its limpidity indefinitely.
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> "How can we fail to believe that by cultivation in the fowl of the attenuated virus we place its body in the state of this filtered liquid which can no longer cultivate the microbe ? The comparison can be pushed still further; for if we filter the *bouillon* containing the microbe in full development, not on the fourth day of culture, but on the second, the filtered liquid will still be able to support the development of the microbe, although with less energy than at the outset. We comprehend, then, that after a cultivation of the modified (*attenué*) microbe in the body of the fowl we may not have removed from all parts of its body the aliment of the microbe. That which remains will permit, then, a new culture, but in a more restricted measure.
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> "This is the effect of a first inoculation ; subsequent inoculations will

remove progressively all the material necessary for the development of the parasite."

In discussing this theory, in a paper published in the *American Journal of the Medical Sciences* (April, 1881), the writer says:

> "Let us see where this hypothesis leads us. In the first place, we must have a material of small-pox, and a material of measles, and a material of scarlet fever, etc., etc. Then we must admit that each of these different materials has been formed in the system and stored up for these emergencies —attacks of the diseases in question—for we can scarcely conceive that they were all packed away in the germ cell of the mother and the sperm cell of the father of each susceptible individual. If, then, these peculiar materials have been formed and stored up during the development of the individual, how are we to account for the fact that no new production takes place after an attack of any one of the diseases in question ?
>
> "Again, how shall we account for the fact that the amount of material which would nourish the small-pox germ, to the extent of producing a case of confluent small-pox, may be exhausted by the action of the attenuated virus (germ) introduced by vaccination ? Pasteur's comparison of a fowl protected by inoculation with the microbe of fowl cholera, with a culture fluid in which the growth of a particular organism has exhausted the pabulum necessary for the development of additional organisms of the same kind, does not seem to me to be a just one, as in the latter case we have a limited supply of nutriment, while in the former we have new supplies constantly provided of the material—food—from which the whole body, including the hypothetical substance essential to the development of the disease germ, was built up prior to the attack. Besides this we have a constant provision for the elimination of effete and useless products.
>
> "This hypothesis, then, requires the formation in the human body, and the retention up to a certain time, of a variety of materials which, so far as we can see, serve no purpose except to nourish the germs of various specific diseases, and which, having served this purpose, are not again formed in the same system, subjected to similar external conditions, and supplied with the same kind of nutriment."

It is unnecessary to discuss this hypothesis any further, inasmuch as it is no longer sustained by Pasteur or his pupils, and is evidently untenable.

The Retention Theory, proposed by Chauveau (1880), is subject to similar objections. According to this view, certain products formed during the development of a pathogenic microörganism in the body of a susceptible animal accumulate during the attack and are subsequently retained, and, being prejudicial to the growth of the particular microörganism which produced them, a second infection cannot occur. Support for this theory has been found by its advocates in the fact that various processes of fermentation are arrested after a time by the formation of substances which restrain the development of the microörganisms to which they are due. But in the case of a living animal the conditions are very different, and it is hard to conceive that adventitious products of this kind could be retained for years, when in the normal processes of nutrition and excretion the tissues and fluids of the body are constantly undergoing change. Certainly the substances which arrest ordinary processes of fermentation by their accumulation in the fermenting liquid, such as alcohol, lactic acid, phenol, etc., would not be so retained. But we cannot speak so positively with reference to the toxic albuminous substances which recent researches have demonstrated to be present in cultures of some of the best known pathogenic bacteria. It is difficult, however, to believe that an individual who has passed through attacks of half a dozen different infectious diseases carries about with him a store of as many different chemical substances produced during these attacks, and sufficient in quantity to prevent the development of the several germs of these diseases. Nor does the experimental evidence relating to the action of germicide and germ-restraining agents justify the view that a substance capable of preventing the development of one microörganism should be without effect upon others of the same class; but if we accept the retention hypothesis we must admit that the inhibiting substance produced by each particular pathogenic germ is effective only in restraining the development of the microbe which produced it in the first instance.

Pasteur discusses this hypothesis in his paper from which we have already quoted, as follows:

> "We may admit the possibility that the development of the microbe, in place of removing or destroying certain matters in the bodies of the fowls, adds, on the contrary, something which is an obstacle to the future development of this microbe. The history of the life of inferior beings authorizes such a supposition. The excretions resulting from vital processes may arrest vital processes of the same nature. In certain fermentations we see antiseptic products make their appearance during, and as a result of, the fermentation, which put an end to the active life of the ferments and arrest the fermentations long before they are completed. In the cultivation of our microbe, products may have been formed the presence of which, possibly, may explain the protection following inoculation.
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> "Our artificial cultures permit us to test the truth of this hypothesis. Let us prepare an artificial culture of the microbe, and after having evaporated it, *in vacuo*, without heat, let us bring it back to its original volume by means of fresh chicken bouillon. If the extract contains a poison for the life of the microbe, and if this is the cause of its failure to multiply in the filtered liquid, the new liquid should remain sterile. Now, this is not the case. We cannot, then, believe that during the life of the parasite certain substances are produced which are capable of arresting its ulterior development."

This experiment of Pasteur appears to be conclusive so far as the particular pathogenic microörganism referred to is concerned; and we may say, in brief, that more recent investigations do not sustain the view that acquired immunity is due to the retention of products such as are formed by pathogenic bacteria in artificial culture media, and which act by destroying these bacteria or restraining their development when they are introduced into the bodies of immune animals.

Moreover, if we suppose that the toxic substances which give pathogenic power to a particular microörganism are retained in the body of an immune animal, we must admit that the animal has acquired a tolerance to the pathogenic action of these toxic substances, for their presence no longer gives rise to any morbid phenomena. And this being the case, we are not restricted to the explanation that immunity depends upon a restraining influence exercised upon the microbe when subsequently introduced.

The Vital Resistance Theory.—Another explanation offers itself, viz., that *immunity depends upon an acquired tolerance to the toxic products of pathogenic bacteria.* This is a view which the writer has advocated in various published papers since 1881. In a paper contributed to the *American Journal of the Medical Sciences* in April, 1881, it is presented in the following language:

> "The view that I am endeavoring to elucidate is that, during a non-fatal attack of one of the specific diseases, the cellular elements implicated which do not succumb to the destructive influence of the poison acquire a tolerance to this poison which is transmissible to their progeny, and which is the reason of the exemption which the individual enjoys from future attacks of the same disease."[1]

In my chapter on "Bacteria in Infectious Diseases," in "Bacteria," published in the spring of 1884, but placed in the hands of the publishers in 1883, I say:

> "It may be that the true explanation of the immunity afforded by a mild attack of an infectious germ disease is to be found in an acquired tolerance to the action of a chemical poison produced by the microörganism, and consequent ability to bring the resources of nature to bear to restrict invasion by the parasite."

The "resources of nature" are referred to in the same chapter as follows:

> "The hypothesis of Pasteur would account for the fact that one individual suffers a severe attack and another a mild attack of an infectious disease, after being subjected to the influence of the poison under identical circumstances, by the supposition that the pabulum required for the development of this particular poison is more abundant in the body of one individual than in the other. The explanation which seems to us more satisfactory is that the vital resistance offered by the cellular elements in the bodies of these two individuals was not the same for this poison. It is well known that in conditions of lowered vitality resulting from starvation, profuse discharges, or any other cause, the power to resist disease poisons is greatly diminished, and, consequently, that the susceptibility of the same individual differs at different times.
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> "From our point of view, the blood, as it is found within the vessels of a living animal, is not simply a culture fluid maintained at a fixed temperature, but under these circumstances is a tissue, the histological elements of which present a certain vital resistance to pathogenic organisms which may be introduced into the circulation.
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> "If we add a small quantity of a culture fluid containing the bacteria of putrefaction to the blood of an animal, withdrawn from the circulation into a proper receptacle and maintained in a culture oven at blood heat, we will find that these bacteria multiply abundantly, and evidence of putrefactive

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[1] "What is the Explanation of the Protection from Subsequent Attacks, resulting from an Attack of Certain Diseases, etc ?" American Journal of the Medical Sciences, April, 1881, p. 376.

decomposition will soon be perceived. But if we inject a like quantity of the culture fluid with its contained bacteria into the circulation of a living animal, not only does no increase and no putrefactive change occur, but the bacteria introduced quickly disappear, and at the end of an hour or two the most careful microscopical examination will not reveal the presence of a single bacterium. This difference we ascribe to the vital properties of the fluid as contained in the vessels of a living animal; and it seems probable that the little masses of protoplasm known as white blood corpuscles are the essential histological elements of the blood, so far as any manifestation of vitality is concerned. *The writer has elsewhere* (1881) *suggested that the disappearance of the bacteria from the circulation, in the experiment referred to, may be effected by the white corpuscles*, which, it is well known, pick up, after the manner of amœbæ, any particles, organic or inorganic, which come in their way. And it requires no great stretch of credulity to believe *that they may, like an amœba, digest and assimilate the protoplasm of the captured bacterium, thus putting an end to the possibility of its doing any harm.*

"In the case of a pathogenic organism we may imagine that, when captured in this way, it may share a like fate if the captor is not paralyzed by some potent poison evolved by it, or overwhelmed by its superior vigor and rapid multiplication. In the latter event the active career of our conservative white corpuscle would be quickly terminated and its protoplasm would serve as food for the enemy. It is evident that in a contest of this kind the balance of power would depend upon circumstances relating to the *inherited* vital characteristics of the invading parasite and of the invaded leucocyte."

In the same chapter the writer quotes from his paper on acquired immunity, published in 1881, as follows :

"The difficulties into which this hypothesis [the exhaustion theory of Pasteur] leads us certainly justify us in looking further for an explanation of the phenomena in question. This explanation is, I believe, to be found in the peculiar properties of the protoplasm, which is the essential framework of every living organism. The properties referred to are the tolerance which living protoplasm may acquire to certain agents which, in the first instance, have an injurious or even fatal influence upon its vital activity ; and the property which it possesses of transmitting its peculiar qualities, inherent or acquired, through numerous generations, to its offshoots or progeny.

"Protoplasm is the essential living portion of the cellular elements of animal and vegetable tissues; but as our microscopical analysis of the tissues has not gone beyond the cells of which they are composed, and is not likely to reveal to us the complicated molecular structure of the protoplasm, upon which, possibly, the properties under consideration depend, it will be best, for the present, to limit ourselves to a consideration of the living cells of the body. These cells are the direct descendants of the pre-existent cells, and may all be traced back to the sperm cell and the germ cell of the parents. Now, the view which I am endeavoring to elucidate is that, during a non-fatal attack of one of the specific diseases, the cellular elements implicated, which do not succumb to the destructive influence of the poison, acquire a tolerance to this poison which is transmissible to their progeny, and which is the reason of the exemption which the individual enjoys from future attacks of the same disease.

"The known facts in regard to the hereditary transmission by cells of acquired properties make it easy to believe in the transmission of such a tolerance as we imagine to be acquired during the attack; and if it is shown by analogy that there is nothing improbable in the hypothesis that such a tolerance is acquired, we shall have a rational explanation, not of heredity and of the mysterious properties of protoplasm, but of the particular result under consideration. The transmission of acquired properties is shown in the budding and grafting of choice fruits and flowers, produced by cultivation, upon the wild stock from which they originated. The acquired properties are transmitted indefinitely; and the same sap which on one twig nourishes a sour crab apple, on another one of the same branch is elaborated into a delicious pippin.

"The tolerance to narcotics—opium and tobacco—and to corrosive poisons —arsenic—which results from a gradual increase of dose, may be cited as an example of acquired tolerance by living protoplasm to poisons which at the outset would have been fatal in much smaller doses.

"The immunity which an individual enjoys from any particular disease must be looked upon as a power of resistance possessed by the cellular elements of those tissues of his body which would yield to the poison in the case of an unprotected person."

This theory of immunity, advanced by the author in 1881, has received considerable support from investigations made since that date, and especially from the experimental demonstration by Salmon, Roux, and others that, as suggested in the paper from which I have quoted, immunity may result from the introduction into the body of a susceptible animal of the soluble products of bacterial growth—filtered cultures.

The theory of vital resistance to the toxic products evolved by pathogenic bacteria is also supported by numerous experiments which show that natural or acquired immunity may be overcome when these toxic products are introduced in excess, or when the vital resisting power of the animal has been reduced by various agencies.

More direct experimental evidence in favor of the view under consideration is that obtained by Beumer in his experiments with sterilized cultures of the typhoid bacillus. He found that after the repeated injection of non-lethal doses mice were able to resist an amount of this toxine which was fatal to animals of the same species not so treated. But, on the other hand, Gamaléia found, in his experiments upon guinea-pigs which had been made immune against the pathogenic action of a spirillum, called by him Vibrio Metschnikovi, that these animals have no increased tolerance for the toxic products of this microörganism. Although immune against infection by the living microbe, they were killed by the same quantity of a sterilized culture as was fatal to guinea-pigs which had not been rendered immune.

Charrin has obtained similar results in experiments with filtered cultures of Bacillus pyocyaneus. Rabbits which had an artificial immunity against the pathogenic action of the bacillus were killed by doses of a sterilized culture such as were fatal to other rabbits of the same size not immune. In subsequent experiments by Charrin and Gameléia "vaccinated" rabbits were found to be even more susceptible to the toxic action of filtered cultures than were those not vaccinated. Metschnikoff (1891) has followed up this line of experiment, and has shown that when considerable amounts of filtered cultures of Bacillus pyocyaneus are injected subcutaneously in rabbits a certain tolerance to the toxic action of the same cultures is established in some instances. But his results do not give any substantial support to the view that immunity depends upon an acquired tolerance to the toxic action of the chemical products contained in cultures of the pathogenic bacteria with which he experimented—Bacillus pyocyaneus and Vibrio Metschnikovi.

In view of the results of experimental researches above recorded, and of other recent experiments which show that, in certain cases at least, acquired immunity depends upon the formation of an antitoxine in the body of the immune animal, we are convinced that the theory of immunity under discussion, first proposed by the writer in 1881, cannot be accepted as a sufficient explanation of the facts in general. At the same time we are inclined to attribute considerable importance to acquired tolerance to the toxic products of pathogenic bacteria as one of the factors by which recovery from an infectious disease is made possible and subsequent immunity established.

The "vital-resistance theory" of the present writer, as set forth in the above-quoted extracts from his published papers, is essentially the same as that advocated by Buchner at a later date (1883). Buchner supposes that during the primary infection, when an animal recovers, a "reactive change" has been produced in the cells of the body which enables it to protect itself from the pathogenic action of the same microörganism when subsequently introduced.

Of course when we ascribe immunity to the "vital resistance" of the cellular elements of the body, we have not explained the *modus operandi* of this vital resistance or "reactive change," but have simply affirmed that the phenomenon in question depends upon some acquired property residing in the living cellular elements of the body. We have suggested that that which has been acquired is a tolerance to the action of the toxic products produced by pathogenic bacteria. But, as already stated, in the light of recent experiments this theory now appears to us to be untenable as a general explanation of acquired immunity.

The Theory of Phagocytosis.—The fact that in certain infectious diseases due to bacteria the parasitic invaders, at the point of inoculation or in the general blood current, are picked up by the leucocytes and in properly stained preparations may be seen in their interior, has been known for some years. In mouse septicæmia—an infectious disease described by Koch in his work on "Traumatic Infectious Diseases," published in 1878—the slender bacilli which are the cause of the disease are found in large numbers in the interior of the leucocytes. Koch says, in the work referred to: "Their relation to the white blood corpuscles is peculiar; they penetrate these *and multiply in their interior.* One often finds that there is hardly a single white corpuscle in the interior of which bacilli cannot be seen. Many corpuscles contain isolated bacilli only; others have thick masses in their interior, the nucleus being still recognizable; while in others the nucleus can be no longer distinguished; and, finally, the corpuscle may become a cluster of bacilli, breaking up at the margin—the origin of which one could not have explained had there been no opportunity of seeing all the intermediate steps between the intact white corpuscle and these masses" (Fig. 78). It will be noted that in the above quotation Koch affirms that the bacilli penetrate the leucocytes and multiply in their interior. Now, the theory of phagocytosis assumes that the bacilli are picked up by the leucocytes and destroyed in their interior, and that immunity depends largely upon the power of these "phagocytes" to capture and destroy living pathogenic bacilli.

The writer suggested this as an hypothesis as long ago as 1881, in a paper read before the American Association for the Advancement of Science, in the following language:

"It has occurred to me that possibly the white corpuscles may have the office of picking up and digesting bacterial organisms which by any means find their way into the blood. The propensity exhibited by the leucocytes for picking up inorganic granules is well known, and that they may be able not only to pick up but to assimilate, and so dispose of, the bacteria which come in their way, does not seem to me very improbable, in view of the fact that amœbæ, which resemble them so closely, feed upon bacteria and similar organisms."[1]

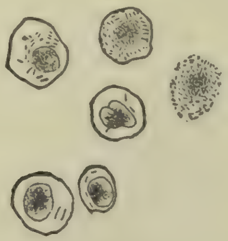

FIG. 78.—Bacillus of mouse septicæmia in leucocytes from blood of mouse (Koch).

At a later date (1884) Metschnikoff offered experimental evidence in favor of this view, and the explanation suggested in the above quotation is commonly spoken of as the *Metschnikoff theory.*

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[1] "A Contribution to the Study of Bacterial Organisms commonly found upon Exposed Mucous Surfaces and in the Alimentary Canal of Healthy Individuals." Illustrated by photomicrographs. Proceedings of the American Association for Advancement of Science, 1881, Salem, 1882, xxx., 83–94. Also in Studies from the Biological Laboratory, Johns Hopkins University, Baltimore, vol. ii., No. 2, 1882.

The observations which first led Metschnikoff to adopt this view were made upon a species of daphnia which is subject to fatal infection by a torula resembling the yeast fungus. Entering with the food, this fungus penetrates the walls of the intestine and invades the tissues. In certain cases the infection does not prove fatal, owing, as Metschnikoff asserts, to the fact that the fungus cells are seized upon by the leucocytes, which appear to accumulate around the invading parasite (chemiotaxis) for this special purpose. If they are successful in overpowering and destroying the parasite the animal recovers; if not, it succumbs to the general infection which results. In a similar manner, Metschnikoff supposes, pathogenic bacteria are destroyed when introduced into the body of an immune animal. The colorless blood corpuscles, which he designates *phagocytes*, accumulate at the point of invasion and pick up the living bacteria, as they are known to pick up inorganic particles injected into the circulation. So far there can be no doubt that Metschnikoff is right. The presence of bacteria in the leucocytes in considerable numbers, both at the point of inoculation and in the general circulation, has been repeatedly demonstrated in animals inoculated with various pathogenic bacteria. The writer observed this in his experiments, made in 1881, in which rabbits were inoculated with cultures of his Micrococcus Pasteuri; and it was this observation which led him to suggest the theory which has since been so vigorously supported by Metschnikoff. But the presence of a certain number of bacteria within the leucocytes does not prove the destructive power of these cells for living pathogenic organisms. As urged by Weigert, Baumgarten, and others, it may be that the bacteria were already dead when they were picked up, having been destroyed by some agency outside of the blood cells. As heretofore stated, we have now experimental evidence that blood serum, quite independently of the cellular elements contained in it in the circulation, has decided germicidal power for certain pathogenic bacteria, and that the blood serum of the rat and other animals which have a natural immunity against anthrax is especially fatal to the anthrax bacillus.

Numerous experiments have been made with a view to determining whether pathogenic bacteria are, in fact, destroyed within the leucocytes after being picked up, and different experimenters have arrived at different conclusions. In the case of mouse septicæmia, already alluded to, and in gonorrhœa, one would be disposed to decide, from the appearance and arrangement of the pathogenic bacteria in the leucocytes, that they are not destroyed, but that, on the other hand, they multiply in the interior of these cells, which in the end succumb to this parasitic invasion. In both of the diseases mentioned we find leucocytes so completely filled with the pathogenic microörganisms that it is difficult to believe that they have all been picked up by a voracious phagocyte, which has stuffed itself to repletion, while numerous other leucocytes from the same source and in the same microscopic field of view have failed to capture a single bacillus or micrococcus. Moreover, the staining of the parasitic invaders, and the characteristic arrangement of the "gonococcus" in stained preparations of gonorrhœal pus, indicate that their vitality has not been destroyed in the interior of the leucocytes or pus cells, and we can scarcely doubt that the large number found in certain cells is due to multiplication *in situ* rather than to an unusual activity of these particular cells. But in certain infectious diseases, and especially in anthrax, the bacilli included within the leucocytes often give evidence of degenerative changes, which would support the view that they are destroyed by the leucocytes, unless these changes occurred before they were picked up, as is maintained by Nuttall and others. We cannot consider this question as definitely settled.

Going back to the demonstrated fact that susceptible animals may be made immune by inoculating them with the toxic products produced during the growth of certain pathogenic bacteria, we may suppose either that immunity results from the continued presence of these toxic products in the body of the inoculated animal, or from a tolerance acquired at the time of the inoculation and subsequently retained—by transmission from cell to cell, as heretofore suggested. Under the first hypothesis—retention theory—immunity may be explained as due to a continued tolerance on the part of the cellular elements of the body to the toxic substances introduced and retained; or to the effect of these retained toxic products in destroying the pathogenic bacteria, or in neutralizing their products when these are subsequently introduced into the body of the immune animal. We cannot understand how toxic substances introduced in the first instance can neutralize substances of the same kind introduced at a later date. There is something in the blood of the rat which, according to Behring, neutralizes the toxic substances present in a filtered culture of the tetanus bacillus; but whatever this substance may be, it is evidently different from the toxic substance which it destroys, and there is nothing in chemistry to justify the supposition last made. Is it, then, by destroying the pathogenic microörganism that these inoculated and retained toxic products preserve the animal from future infection? Opposed to this supposition is the fact that the blood of an animal made immune in this way, when removed from the body, does not prove to have increased germicidal power as compared with that of a susceptible animal of the same species. Again, these same toxic substances in cultures of the anthrax bacillus, the tetanus bacillus, the diphtheria bacillus, etc., do not destroy the pathogenic germ after weeks or months of exposure. And when we inoculate a susceptible animal with a virulent culture of one of these microörganisms, the toxic substances present do not prevent the rapid development of the bacillus ; indeed, instead of proving a germicide, they favor its development, which is more abundant and rapid than when attenuated cultures containing less of the toxic material are used for the inoculation. In view of these facts we are unable to adopt the view that acquired immunity results from the direct action of the products of bacterial growth, introduced and retained in the body of the immune animal, upon the pathogenic microörganism when subsequently introduced or upon its toxic products.

But there is another explanation which, although it may appear *a priori* to be quite improbable, has the support of recent experimental evidence. This is the supposition that *some substance is formed in the body of the immune animal which neutralizes the toxic products of the pathogenic microörganism.* How the presence of these toxic products in the first instance brings about the formation of an "antitoxin" by which they are neutralized is still a mystery; but that such a substance is formed appears to be proved by the experiments of Ogata, Behring and Kitasato, Tizzoni and Cattani, G. and F. Klemperer, and others.

Ogata and Jasuhara, in a series of experiments made in the Hygienic Institute at Tokio (1890), discovered the important fact that the blood of an animal immune against anthrax contains some substance which neutralizes the toxic products of the anthrax bacillus. When cultures were made in the blood of dogs, frogs, or of white rats, which animals have a natural immunity against anthrax, they were found not to kill mice inoculated with them. Further experiments showed that mice inoculated with virulent anthrax cultures did not succumb to anthrax septicæmia if they received at the same time a subcutaneous injection of a small quantity of the blood of an immune animal. So small a dose as one drop of frog's blood or one-half drop of dog's blood proved to be sufficient to protect a mouse from the fatal effect of an anthrax inoculation. And the protective inoculation was effective when made as long as seventy-two hours before or five hours after infection with an anthrax culture. Further, it was found that mice which had survived anthrax infection as a result of this treatment were immune at a later date (after several weeks) when inoculated with a virulent culture of the anthrax bacillus.

Behring and Kitasato have obtained similar results in their experiments upon tetanus and diphtheria, and have shown that the blood of an immune animal, added to virulent cultures before inoculation into susceptible animals, neutralizes the pathogenic power of these cultures.

They have shown by experiment that the blood of a rabbit which has an acquired immunity against tetanus, mixed with the virulent filtrate from a culture of the tetanus bacillus, neutralizes its toxic power. One cubic centimetre of this filtrate was mixed with five cubic centimetres of serum from the blood of an immune rabbit and allowed to stand for twenty-four hours ; 0.2 cubic centimetre of this injected into a mouse was without effect, while 0.0001 cubic centimetre of the filtrate without such admixture was infallibly fatal to mice. The mice inoculated with this mixture remained immune for forty to fifty days, after which they gradually lost their immunity. The blood or serum from an immune rabbit, when preserved in a dark, cool place, retained its power of neutralizing the tetanus toxalbumin for about a week, after which time it gradually lost this power. The blood of chickens, which have a natural immunity against tetanus, was found not to have a similar power. Behring and Kitasato have also shown that the serum of a diphtheria-immune rabbit destroys the potent toxalbumin in diphtheria cultures. It does not, however, possess any germicidal power against the diphtheria bacillus.

Ogata, in 1891, reported that he had succeeded in isolating from the blood of dogs and of chickens a substance to which he ascribes the natural immunity of these animals from certain infectious diseases, and the power of their blood to protect susceptible animals from the same diseases. This substance is soluble in water and in glycerin, but insoluble in alcohol or ether, by which it is precipitated without being destroyed. Its activity is neutralized by acids, but not by weak alkaline solutions. Ogata supposes the substance isolated by him to be the active agent in blood serum by which certain pathogenic bacteria are destroyed, as shown by the experiments of Nuttall, Buchner, and others. Hankin had previously isolated an albuminoid substance from the spleen and blood of the rat, to which he ascribed the immunity of this animal from anthrax. This substance, according to the author named, is a globulin; it is insoluble in alcohol and in distilled water, and does not dialyze.

Tizzoni and Cattani ascribe the protection of animals which have acquired an immunity against tetanus to the presence of an albuminous substance which they call the tetanus-antitoxin. This they have isolated from the blood of immune animals. They arrive at the conclusion that it is a globulin, or a substance which is carried down with the globulin precipitate, and that it is different from the globulin, above referred to, obtained by Hankin from animals immune against anthrax.

G. and F. Klemperer, in 1891, published an important memoir in which they gave an account of their researches relating to the question of immunity, etc., in animals subject to the form of septicæmia produced by the Micrococcus pneumoniæ crouposæ. They were able to produce immunity in susceptible animals by introducing into their bodies filtered cultures of this micrococcus, and proved by experiment that this immunity had a duration of at least six months. They arrived at the conclusion that the immunity induced by injecting filtered cultures is not directly due to the toxic substances present in these cultures, but that they cause the production in the tissues of an antitoxin which has the power of neutralizing their pathogenic action. The toxic substance present in cultures of the "diplococcus of pneumonia" they call "pneumotoxin"; the substance produced in the body of an artificially immune animal, by which this pneumotoxin is destroyed if subsequently introduced, they call "anti-pneumotoxin."

Emmerich, in a communication made at the meeting of the International Congress for Hygiene and Demography, in London, reported results which correspond with those of G. and F. Klemperer so far as the production of immunity is concerned, and also gave an account of experiments made by Dönissen in which the injection of twenty to twenty-five cubic centimetres of blood or expressed tissue juices, filtered through porcelain, from an immune rabbit into an unprotected rabbit, subsequently to infection with a bouillon culture of "diplococcus pneumoniæ," prevented the development of fatal septicæmia. Even when the injection was made twelve to fifteen hours after infection, by inhalation, the animal recovered.

Emmerich and Mastraum had previously reported similar results in experiments made upon mice with the Bacillus erysipelatos suis (rothlauf bacillus). White mice are very susceptible to the pathogenic action of this bacillus. But mice which, subsequently to infection, were injected with the expressed and filtered tissue juices of an immune rabbit, recovered, while the control animals succumbed. According to Emmerich, the result in these experiments was due to a destruction of the pathogenic bacilli in the bodies of the infected animals; and the statement is made that at the end of eight hours after the injection of the expressed tissue juices all bacilli in the body of the infected animal were dead. The same liquid did not, however, kill the bacilli when added to cultures external to the body of an animal. The inference, therefore, seems justified that the result depends, not upon a substance present in the expressed juices of an immune animal, but upon a substance formed in the body of the animal into which these juices are injected.

We have, however, an example of induced immunity in which the result appears to depend directly upon the destruction of the pathogenic microörganism in the body of the immune animal. In guinea-pigs which have an acquired immunity against Vibrio Metschnikovi the blood serum has been proved to possess decided germicidal power for this "vibrio," whereas it multiplies readily in the blood serum of non-immune guinea-pigs (Behring and Nissen).

There is experimental evidence that animals may acquire an artificial immunity against the toxic action of certain toxalbumins from other sources than bacterial cultures. Thus Sewell (1887) has shown that a certain degree of tolerance to the action of rattlesnake venom may be established by inoculating susceptible animals with small doses of the "hemialbumose" to which it owes its toxic potency. These results have been confirmed by the more recent experiments of Calmette (1894) and of Fraser (1895). In his paper detailing the results of his experiments the first-named author says:

> "Animals may be immunized against the venom of serpents either by means of repeated injections of doses at first feeble and progressively stronger, or by means of successive injections of venom mixed with certain chemical substances, among which I mention especially chloride of gold and the hypochlorites of lime or of soda.
>
> "The serum of animals thus treated is at the same time preventive, antitoxic, and therapeutic, exactly as is that of animals immunized against diphtheria or tetanus.
>
> "If we inoculate a certain number of rabbits, under the skin of the thigh, with the same dose, one milligramme of cobra venom for example, and if we treat all of these animals with the exception of some for control, by subcutaneous or intraperitoneal injections of the serum of rabbits immunized against four milligrammes of the same venom, all of the control animals not treated will die within three or four hours, while all of the animals will recover which receive five cubic centimetres of the therapeutic serum within an hour after receiving the venom."

In this connection we may remark that there is some evidence to show that persons who are repeatedly stung by certain poisonous insects—mosquitoes, bees—acquire a greater or less degree of immunity from the distressing local effects of their stings.

Ehrlich, of Berlin, in 1891, reported his success in establishing immunity in guinea-pigs against two toxalbumins of vegetable origin: one—ricin—from the castor-oil bean (Ricinus communis), the other—abrin—from the jequirity bean. The toxic potency of ricin is somewhat greater than that of abrin, and it is estimated by Ehrlich that one gramme of this substance would suffice to kill one and a half millions of guinea-pigs. When injected beneath the skin, in dilute solution, it produces intense local inflammation, resulting in necrosis of the tissues. Mice are less susceptible than guinea-pigs and are more easily made immune. This is most readily effected by giving them small and gradually increasing doses with their food. As a result of this treatment the animal resists subcutaneous injections of two hundred to four hundred times the fatal dose for animals not having this artificial immunity. The fatal dose of abrin is about double that of ricin. When injected into mice in the proportion of one cubic centimetre to twenty grammes of body weight a solution of one part in one hundred thousand of water proved to be a fatal dose. The local effects are also less pronounced when solutions of abrin are used; they consist principally in an extensive induration of the tissues around the point of injection and a subsequent falling off of the hair over this indurated area. When introduced into the conjunctival sac, however, abrin produces a local inflammation in smaller amounts than ricin, a solution of 1:800 being sufficient to cause a decided but temporary conjunctivitis. Solutions of 1:50 or 1:100 of either of these toxalbumins, introduced into the eye of a mouse, give rise to a panophthalmitis which commonly results in destruction of the eye. But in mice which have been rendered immune by feeding them for several weeks with food containing one of these toxalbumins, no reaction follows the introduction into the eye of the strongest possible solution, or of a paste made by adding abrin to a little ten-per-cent salt solution. Ehrlich gives the following explanation of the remarkable degree of immunity established in his experiments by the method mentioned:

"All of these phenomena depend, as may be easily shown, upon the fact that the blood contains a body—antiabrin—which completely neutralizes the action of the abrin, probably by destroying this body."

In a more recent paper Ehrlich has given an account of subsequent experiments which show that the young of mice which have an acquired immunity for these vegetable toxalbumins may acquire immunity from the ingestion of the mother's milk; and also that immunity against tetanus may be acquired in a very brief time by young mice through their mother's milk. In his tetanus experiments Ehrlich used blood serum from an immune horse to give immunity to the mother mouse when her young were already seventeen days old. Of this blood serum two cubic centimetres were injected at a time on two successive days. The day after the first injection one of the sucklings received a tetanus inoculation by means of a splinter of wood to which spores were attached. The animal remained in good health, while a much larger control mouse inoculated in the same way died of tetanus at the end of twenty-six hours. Other sucklings, inoculated at the end of forty-eight and of seventy-two hours after the mother had received the injection of blood serum, likewise remained in good health, while other control mice died.

The possibility of conferring immunity by means of the milk of an immune animal is further shown by the experiments of Brieger and Ehrlich (1892). A female goat was immunized against tetanus by the daily injection of "thymus-tetanus bouillon." The dose was gradually increased from 0.2 cubic centimetre to 10 cubic centimetres. At the end of thirty-seven days a mouse, which received 0.1 cubic centimetre of the milk of this goat in the cavity of the abdomen, proved to be immune against tetanus. Further experiments gave a similar result, even when the milk of the goat was not injected into the peritoneal cavity of the mouse until several hours after inoculation with a virulent culture of the tetanus bacillus.

When the casein was separated the milk retained its full immunizing activity, and by concentration *in vacuo* a thick milk was obtained which had a very high immunization value—0.2 cubic centimetre of this milk protected a mouse against forty-eight times the lethal dose of a tetanus culture.

In a subsequent communication (1893) Brieger and Ehrlich describe their method of obtaining the antitoxin of tetanus from milk in a more concentrated form. They found by experiment that it was precipitated by ammonium sulphate and magnesium sulphate. From twenty-seven to thirty per cent of ammonium sulphate added to milk caused a precipitation of the greater part of the antitoxin. This precipitate was dissolved in water, dialyzed in running water, then filtered and evaporated in shallow dishes at 35° C. in a vacuum. One litre of milk from an immune goat gave about one gramme of a transparent, yellowish-white precipitate, which contained fourteen per cent of ammonium sulphate. This precipitate had from four hundred to six hundred times the potency of the milk from which it was obtained in neutralizing the tetanus toxin.

In a still later communication (1893) Brieger and Cohn give an improved method of separating the antitoxin from the precipitate thrown down with ammonium sulphate. The finely pulverized precipitate is shaken up with pure chloroform, and when this is allowed to stand the antitoxin rises to the surface while the ammonium salt sinks to the bottom. By filling the vessel to the margin with chloroform, the antitoxin floating on the surface can be skimmed off, after which it quickly dries. By this method the considerable loss which occurred in the dialyzer, used in the previously described method, is avoided.

A most interesting question presents itself in connection with the discovery of the antitoxins. Does the animal which is immune from the toxic action of any particular toxalbumin also have an immunity for other toxic proteids of the same class? The experimental evidence on record indicates that it does not. In Ehrlich's experiments with ricin and abrin he ascertained that an animal which had been made immune against one of these subtances was quite as susceptible to the toxic action of the other as if it did not possess this immunity, *i.e.*, the antitoxin of ricin does not destroy abrin, and *vice versa.* As an illustration of the fact, he states that in one experiment a rabbit was made immune for ricin to such an extent that the introduction into its eye of this substance in powder produced no inflammatory reaction; but the subsequent introduction of a solution of abrin, of 1 to 10,000, caused a violent inflammation.

Evidently these facts are of the same order as those relating to immunity from infectious diseases, and, taken in connection with the experimental data previously referred to, give strong support to the view that the morbid phenomena in all diseases of this class are due to the specific toxic action of substances resembling the toxalbumins already discovered ; and that acquired immunity from any one of these diseases results from the formation of an antitoxin in the body of the immune animal.

Hankin calls these substances produced in the bodies of immune animals "defensive proteids," and proposes to classify them as follows : First, those occurring naturally in normal animals, which he calls *sozins ;* second, those occurring in animals that have acquired an artificial immunity—these he calls *phylaxins.* Each of these classes of defensive proteids is further subdivided into those which act upon the pathogenic microörganism itself and those which act upon its toxic products. These subclasses are distinguished by the prefixes *myco* and *toxo* attached to the class name.

In accordance with this classification a *mycosozin* is a defensive proteid, found in the body of a normal animal, which has the power of destroying bacteria.

A *toxosozin* is a defensive proteid, found in the body of a normal animal, which has the power of destroying the toxic products of bacterial growth.

A *mycophylaxin* is a defensive proteid produced in the body of an animal which has an acquired immunity for a given infectious disease, which has the power of destroying the pathogenic bacteria to which the disease is due.

A *toxophylaxin* is a defensive proteid produced in the body of an animal which has an acquired immunity for a given infectious disease, which has the power of destroying the toxic products of the pathogenic bacteria to which the disease is due.

Buchner had previously proposed the name "alexins" for these defensive proteids.

The importance of the experimental evidence above referred to in explaining the phenomena of natural and acquired immunity is apparent. The facts stated also suggest a rational explanation of recovery from an attack of an acute infectious disease. But the idea that during such an attack an antidote to the disease poison is developed in the tissues is yet so novel, and the experimental evidence in support of this view is of such recent date, that it would be premature to accept this explanation as applying to immunity in general. It seems difficult to believe that an individual who has passed through attacks of measles, mumps, whooping cough, scarlet fever, small-pox, etc., has in his blood or tissues a store of the antitoxin of each of these diseases, formed during the attack and retained during the remainder of his life, or continuously produced so long as the immunity lasts. Moreover, in those diseases to which the experimental evidence above recorded relates—diphtheria, tetanus, pneumonia—as they occur in man, no lasting immunity has been shown to result from a single attack, and in this regard they do not come into the same class with the eruptive fevers and other diseases in which a single attack usually protects during the lifetime of the individual.

In those instances in which acquired immunity has been shown to be due to the production in the body of the immune animal of an antitoxin, it is still uncertain whether there is a continuous production of the protective proteid, or whether that formed during the attack remains in the body during the subsequent immunity. The latter supposition appears at first thought improbable ; but when we remember that the protective proteids which have been isolated by Hankin from the blood and spleen of rats, and by Tizzoni and Cattani from the blood of animals made immune against tetanus, *do not dialyze*, it does not seem impossible that these substances might be retained indefinitely within the blood-vessels. On the other hand, the passage of the tetanus antitoxin into the mother's milk, as shown by Ehrlich's experiments upon mice, indicates a continuous supply, otherwise the immunity of the mother would soon be lost.

The writer has obtained (May, 1892) experimental evidence that the blood of vaccinated, and consequently immune, calves contains something which neutralizes the specific virulence of vaccine virus, both bovine and humanized. Four drops of blood serum from a calf which had been vaccinated two weeks previously, mixed with one drop of liquid lymph recently collected in a capillary tube, after contact for one hour was used to vaccinate a calf; the same animal was also vaccinated with lymph, preserved on three quills, which was mixed with four drops of serum from the immune calf and left for one hour. The result of these vaccinations was entirely negative, while vaccinations upon the same calf made with virus from the same source, and mixed with the same amount of blood serum from a non-immune calf, gave a completely successful and typical result.

The experimental evidence detailed shows that in certain diseases *acquired immunity depends upon the formation of antitoxins in the bodies of immune animals.* As secondary factors it is probable that tolerance to the toxic products of pathogenic bacteria and phagocytosis have considerable importance, but it is evident that the principal rôle cannot be assigned to these agencies.

As a rule the antitoxins have no bactericidal action; but it has been shown by the experiments of Gamaléia, Pfeiffer, and others, that in animals which have an acquired immunity against the spirillum of Asiatic cholera and against spirillum Metschnikovi, there is a decided increase in the bactericidal power of the blood serum, and that immunity probably depends upon this fact.

The researches of Metschnikoff upon hog cholera, of Issaef upon pneumonia, and of Sanarelli upon typhoid fever indicate that the immunity conferred upon susceptible animals by protective inoculations is not due to an antitoxin but to a substance present in the blood of immune individuals which acts directly upon the pathogenic microörganism, as is the case in cholera-immune animals. The animals immunized are said to be quite as sensitive to the action of the bacterial poisons as are those which have not received protective inoculations. "Their serum does not protect against the toxin, but against the microbe" (Roux).

According to Buchner (1894) the antitoxins are to be regarded not as reactive products developed in the body of the immune animal, but as modified, changed, and "*entgiftete*" products of the specific bacterial cells. He insists that they do not neutralize the toxins by direct contact, but only through the medium of the living organism. This explanation scarcely appears tenable in view of the experimental evidence, and the fact that the antitoxin of tetanus escapes in considerable quantity with the milk of an immune goat without, apparently, diminishing the immunity of the animal. In the immunity against the toxic action of the vegetable toxalbumins—ricin and abrin—as shown by Ehrlich's experiments, there are no "products of bacterial cells" introduced with the pure toxalbumin from the castor bean or the jequirity bean; and we have sufficiently numerous experiments to show that immunity, with the presence of antitoxins in the blood, may be induced by precipitated and purified toxalbumins from filtered cultures. Several of the experimenters, also, have reported that the toxins from bacterial cultures are neutralized *in vitro* by blood-serum from an immune animal, or by the precipitated antitoxin from such serum after contact for a certain number of hours. If they are correct in the statement that a certain time is required after the antitoxin has been brought in contact with the toxin, in order that the latter may be neutralized, as shown by injection of the mixture into a susceptible animal, then we must admit that this neutralizing effect occurs outside of the body of the animal, as has been generally assumed.

The experiments of Vaillard are also opposed to Buchner's view. He reports that in a rabbit immunized against tetanus, "a volume of blood equal to the total amount which circulates in its body may be withdrawn without diminishing, in an appreciable manner, the antitoxic power of its serum. Therefore the antitoxin must be reproduced as fast as it is withdrawn." The author from whom we have just quoted (Roux) also reports the results of experiments which show that the antitoxic value of the serum of a rabbit immunized against tetanus does not bear a direct relation to the quantity of the tetanus toxin introduced, but depends also upon the method adopted. When a few large doses are given the result is far less favorable than that obtained by giving the same amount in repeated small doses. The serum of an animal immunized by thirty-three small doses was found to neutralize, *in vitro*, 150 parts of toxin, while that of an animal which received the same amount in nine doses only neutralized 25 parts of the same toxin. On the other hand we have experiments which indicate that the supposed neutralization of a toxin by an antitoxin *in vitro* is not really a chemical neutralization. Thus Buchner found in his experiments with the tetanus toxin and antitoxin, in a dry powder, that when mixed in a certain proportion and injected into white mice no tetanic symptoms were induced. But the same mixture gave rise to distinct tetanic symptoms in guinea-pigs, showing that the inference that the toxin had been neutralized *in vitro*, based upon the experiment on mice, would have been a mistake. And certain observations made by Roux and Vaillard seem to give support to the view that neutralization does not occur *in vitro*, but that the result depends upon some physiological reaction induced by the antitoxin within the body of the living animal. These bacteriologists found that when the antitoxin was apparently in excess, tetanic symptoms could be induced in susceptible animals if they had been in any way exhausted prior to the injection of the mixture of toxin and antitoxin; and that the same result followed when their resisting power had been reduced by injecting into them at the same time filtered cultures of other bacteria.

In this connection the results reported by Calmette, Phisalix, and Bertrand are of interest. These investigators found that when the antitoxin of snake-poison was mixed with this venom in a proportion which neutralized its toxic properties, as shown by experimental inoculations, and the mixture then heated to 70° C., by which temperature the antitoxin is destroyed, subsequent inoculations showed that the toxin was still active.

The experiments of Stern (1894) show that the typhoid bacillus not only grows in blood-serum from a typhoid convalescent, which has been proved to neutralize its pathogenic effects when injected into a susceptible animal, but also that its toxic products are developed in this culture medium. From this Stern concludes that the serum must in some way act upon the infected animal, causing changes which enable it to resist infection, rather than upon the bacillus or upon its toxic products directly. It has also been shown by Behring (1890) for the diphtheria bacillus, by Vaillard for the tetanus bacillus (1892), and by Issaeff (1893) for the micrococcus of pneumonia, that these several pathogenic microörganisms may be cultivated in the blood-serum of animals immunized for the diseases which they produce.

In a paper published in 1897, Ehrlich advanced his "side-chain" (seitenkette) theory. He considers the individual cells of the body to be analogous, in a certain sense, to complex organic substances, and that they consist essentially of a central nucleus to which secondary atom-groups having distinct physiological functions are attached by "side chains"—such as chemists represent in their attempts to illustrate the reactions which occur in the building up or pulling down of complex organic compounds. The cell-equilibrium is supposed to be disturbed by injury to any of its physiological atom-groups—as by a toxin—and this disturbance results in an effort at compensatory repair during which plastic material in excess of the amount required is generated and finds its way into the blood. This Ehrlich regards as the antitoxin, which is capable of neutralizing the particular toxin to which it owes its origin, if this is subsequently introduced into the blood. In this theory a specific combining relation is assumed to exist between various toxic substances and the secondary atom-groups of certain cellular elements of the body. The atom-groups which, in accordance with this theory, combine with the toxin of any particular disease germ, Ehrlich calls the "toxophoric side chain." Immunity, according to Ehrlich, is either "active" or "passive." Passive immunity results from the introduction of the immunizing substance from an immunized animal into the circulation of a non-immune animal, *e.g.*, the use of diphtheria antitoxin as a prophylactic. This passive immunity is more transient than the active immunity which results from an attack of an infectious disease, from inoculations with living vaccines, or from repeated injections of increasing doses of the toxins of pathogenic bacteria. Ehrlich's explanation of immunity, however probable it may appear, can hardly be said to rest upon a substantial experimental foundation, and we must admit that the exact source and method of production of the antitoxins in the animal body, and their mode of action, are still undetermined; and, for the present, we must be satisfied with the knowledge that in some way these so-called antitoxins, which have been proved to be present in the blood-serum of immune animals, protect these animals from infection by pathogenic bacteria. And that when transferred to susceptible animals they confer upon them a temporary immunity; or if introduced after infection, may neutralize the pathogenic action of the toxins produced by specific "disease germs."

Finally, there is experimental evidence to show that immunity from the pathogenic action of certain bacteria may be produced by previous injections of cultures of other bacteria (sterilized or otherwise), and even by the injection of the blood-serum of normal individuals or of other substances.

Pasteur, in 1880, communicated to the French Academy of Sciences the results of experiments which led him to the conclusion that fowls which had an acquired immunity against chicken cholera also had an immunity against anthrax. Roux has reported that the blood-serum of a horse which has been immunized against tetanus neutralizes the toxic power of cobra poison. But the contrary effect is not produced—*i.e.*, the blood-serum of an animal immunized against the cobra poison does not neutralize the tetanus toxalbumin. The statement is also made that the blood-serum of a rabbit which has been made immune against hydrophobia will protect a susceptible animal against the cobra venom in doses four or five times as large as the usually lethal dose. Also that rabbits which have been immunized against snake-poison are less susceptible to the toxic effects of abrin, and the reverse—*i.e.*, antiabrin neutralizes, to some extent at least, the toxic action of snake-poison.

The writer, in his "Report on the Etiology and Prevention of Yellow Fever" (1890), gives, on pp. 196 and 197, experimental evidence which shows that the injection into the peritoneal cavity of rabbits of cultures of Bacillus pyocyaneus or of Bacillus gracilis protected the animals from the fatal results of subsequent injections of my bacillus X, which was extremely fatal to rabbits when injected into the cavity of the abdomen in doses of 1 or 2 c.c. In referring to these experiments I say: "The evidence favors the view that death results from peritonitis (and toxæmia?) induced by intra-peritoneal injections, and that *a tolerance on the part of the peritoneum may*

PLATE IV.

Figs. 1, 2, and 3.—Leucocytes from the spleen of an inoculated monkey, containing Spirillum Obermeieri. (Soudakewitch.)

Figs. 4 and 5.—Leucocytes ("macrophages") from a preparation of muscle from a pigeon which succumbed to an anthrax inoculation. In Fig. 4 the bacilli are deeply stained; in Fig. 5 they are pale. (Metschnikoff.)

Fig. 6.—Leucocyte from a frog seventy-two hours after the injection of anthrax spores. (Trapeznikoff.)

Figs. 7 and 8.—Leucocytes from a chicken four hours after the injection of anthrax spores. (Trapeznikoff.)

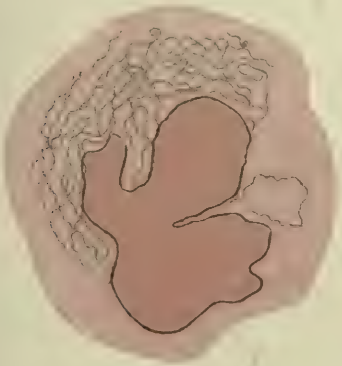
Fig. 1.

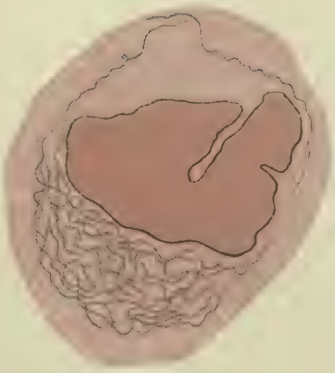
Fig. 2.

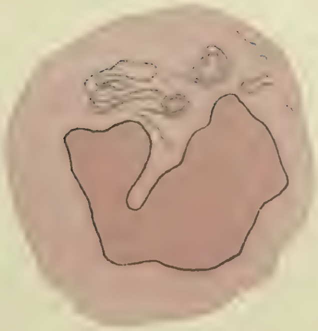
Fig 3.

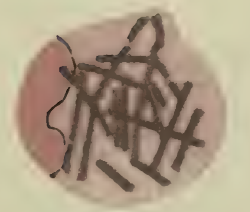
Fig. 4.

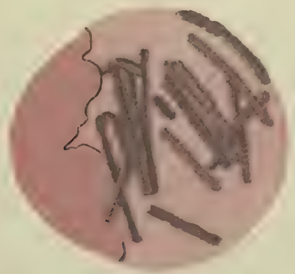
Fig. 5.

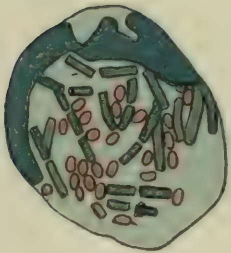
Fig. 6.

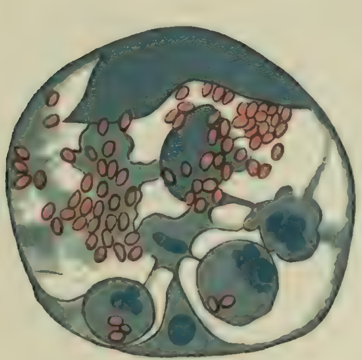
Fig. 7.

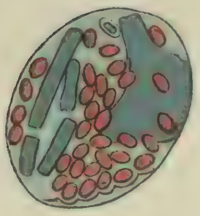
Fig. 8.

PHAGOCYTES.

be established by the injection of certain other bacilli, or of sterilized cultures of bacillus X."

This corresponds with facts subsequently developed by Issaeff (1894) in his experiments with reference to immunity in guinea-pigs against cholera cultures injected into the cavity of the abdomen. He found that a certain degree of immunity was established by the previous injection of blood-serum from normal individuals, and also of various acids, alkalies, and neutral liquids. The immunity produced in this way was, however, feeble and temporary, and could not properly be considered as identical with that produced by inoculations with attenuated cultures which give rise to a mild attack of a specific disease.

Cesaris-Demel and Orlandi have reported (1894) their success in immunizing animals against infection by the typhoid bacillus by means of sterilized cultures of Bacillus coli communis, and the reverse.

While this chapter relates especially to acquired immunity from infectious diseases, and this immunity has been shown to depend, in a number of these diseases at least, upon the development of antitoxins in the body of the immune animal, it may be worth while to refer briefly, before closing, to some examples of acquired immunity of a different order. We refer to the tolerance to extremes of heat and cold which may be established by habitual exposure, and, more especially, to the tolerance to narcotics and irritant poisons, which is very remarkable and has never been explained in a satisfactory manner. Samuel (1892) has presented experimental evidence which shows that the local inflammation which results from the application of croton-oil to the ear of a rabbit does not occur when a second application is made to the same ear after recovery from the effects of the first. That a tolerance may be acquired to comparatively large doses of arsenic is well known, and the tolerance which the victims of drug habits acquire to enormous doses of narcotics is a matter of daily observation. In the writer's paper on acquired immunity, published in 1881, an attempt was made to account for acquired immunity in infectious diseases as analogous to the immunity to drugs just referred to; but the experimental evidence presented in the present chapter shows that the analogy has no scientific foundation in the absence of any evidence that there is an antitoxin of morphia, of cocaine, of narcotin, etc., in the blood of the *habitués* of these drugs.

IV.

PROTECTIVE INOCULATIONS.

ANTHRAX.

The discovery of the anthrax bacillus by Davaine (1863), and the demonstration of its etiological relation to the disease with which it is associated, by the researches of Pasteur, Toussaint, Koch (1878–1881) and other pioneers in this field of investigation, constitute the foundation of our present knowledge of bacteriology and of the practical results attained in protective inoculations and serum-therapy. And a review of the literature relating to the anthrax bacillus would show, in a most interesting manner, the successive steps by which we have arrived at the important results which have gone so far toward establishing medicine upon a scientific basis. In the present volume, however, we must confine our attention to those investigations which relate directly to the subject in hand.

Toussaint, a pioneer in researches relating to protective inoculations, has a short paper in the *Comptes-Rendus of the French Academy of Sciences* of July 12th, 1880, entitled "Immunity from Anthrax ("charbon") Acquired as a Result of Protective Inoculations."

In this paper he announces his discovery of the important fact that the anthrax bacillus does not form spores in the tissues or liquids of the body of an infected animal, but multiplies alone by binary division—"*sa multiplication se fait toujours par une division du mycélium.*"

In the same communication he reports his success in conferring immunity upon five sheep by means of protective inoculations, and also upon four young dogs. We must therefore accord him the priority in the publication of experimental data demonstrating the practicability of accomplishing this result.

Toussaint does not give his method in the communication above referred to, but the following quotation from a communication made to the Academy of Sciences on March 19th, 1881, by Pasteur, shows the method, and at the same time demonstrates the fact that Toussaint was the first to produce immunity by the use of sterilized cultures. Pasteur says:

> "By inoculating sheep either with defibrinated blood from an animal dead of anthrax, after filtration through several thicknesses of paper, or

> with the same blood defibrinated and subjected to 55° C. for ten minutes, according to Toussaint, these sheep subsequently resist inoculations with anthrax blood. . . . The bacillus, according to Toussaint, deposits in the blood of animals in which it multiplies a substance which may become its own vaccine. By filtration while cold in one case, by a temperature of 55° C. in the other, the bacillus is said to be removed or killed; so that the inoculation of filtered or heated blood introduces into the animal inoculated vaccinal matter deprived of bacteria."

After thus stating Toussaint's method and explanation Pasteur proceeds to raise objections against this method, the principal of which are that the anthrax bacillus is not killed by exposure to a temperature of 55° C. for ten minutes, and that inoculation with a virus prepared in this way would result in a considerable mortality among the animals inoculated, although those surviving the inoculation would be protected.

In a communication made to the French Academy of Sciences, September 27th, 1880, Pasteur gave an account of an experiment made July 14th, 1879, upon two cows, which in connection with a subsequent experiment, made August 6th, 1880, upon four cows, led him to the conclusion that a single attack of anthrax protects from subsequent attacks. He says in the paper referred to:

> "On the 15th of September, 1880, two cows, A and C, which had been very ill as a result of the first inoculation, made August 6th, were reinoculated on the left side, that is to say, on the side opposite the first inoculation. We used five drops of culture of the bacillus of anthrax ('*bactéridies du charbon*'). The following days there was no perceptible œdema and no elevation of temperature in either cow. The question is then resolved: a single attack protects ('*le charbon ne récidive pas*')."

The next important steps in the line of experimental research leading to protective inoculations in the disease under consideration were reported by Pasteur in his communication to the French Academy made at the séance of February 28th, 1881 (with the collaboration of Chamberland and Roux), entitled "De l'atténuation des virus et de leur retour à la virulence." In this connection Pasteur announces his discovery of the fact that when cultivated at a temperature of 42° to 43° C., the anthrax bacillus no longer forms spores and rapidly loses its virulence. He says:

> "As regards its virulence, the extraordinary fact has been ascertained that the bacillus is no longer virulent after it has been kept for eight days at a temperature of 42° to 43° C.; at least its cultures are inoffensive for the guinea-pig, the rabbit, and the sheep, three species of animals which are very susceptible to anthrax. We are able, then, not only to attenuate virulence, but to effect its complete extinction, by a simple method of cultivation.
>
> "Before the extinction of its virulence the microbe of charbon passes through the intermediate degrees of attenuation, and, on the other hand, as happens also with the microbe of fowl cholera, each of these degrees of vir-

> ulence may be reproduced by cultivation. Finally, as shown in one of our recent communications, since one attack of anthrax protects, each one of our attenuated microbes of charbon constitutes a vaccine for the microbe of superior virulence; that is to say, a virus suitable to produce a more benign malady. What, then, is more easy than to find among these a virus suitable to give anthrax to sheep, cows, or horses, without causing them to perish, and capable of preserving them from a subsequent fatal attack? We have already practised this operation upon sheep with great success."

At the end of this important communication Pasteur says:

> "I concluded my communication of October 26th by remarking that the attenuation of virus by the influence of the air is probably one of the factors in the extinction of great epidemics. The facts just recorded, in their turn, may serve to explain the so-called spontaneous appearance of these scourges. An epidemic which has been terminated by the attenuation of its virus may be relighted by the reinforcement of this virus under certain influences. The accounts which I have read of the spontaneous appearance of the plague appear to me to offer examples of this. The plague is a virulent malady which prevails in certain countries. In all of these countries its attenuated virus probably exists, ready to take its active form when the necessary conditions as to climate, famine, and distress again prevail. There are other virulent maladies which appear spontaneously in all countries, such as camp typhus. Without doubt the germs of the microbes which cause these diseases are everywhere distributed. Man carries them about him, or in his intestine, without great damage, but ready, nevertheless, to become dangerous when, as a result of certain conditions or of successive development upon the surface of wounds, in bodies enfeebled or otherwise, their virulence is progressively reinforced. And from this point of view virulence appears to us under a new light which is somewhat disquieting for humanity, unless nature, in the evolution which has occurred during the past centuries, has already encountered all possible occasions for the production of virulent or contagious diseases, an assumption which seems very improbable.
>
> "What is an inoffensive microscopic organism for man or for a given animal? It is an organism which cannot develop in our body or in that of the animal; but nothing proves that if this microscopic organism should penetrate into some other of the thousands of species of the creation, it could not invade it and cause it to become sick. Its virulence, then, reinforced by passing through a series of individuals of this species, might become such that it could invade man or one of the domestic animals. By this means new contagions may be created. I am disposed to believe that it is in this way that, in the course of ages, have appeared small-pox, syphilis, the plague, yellow fever, etc."

This broad induction has received considerable support from more recent researches, which show that the typhoid bacillus, the cholera spirillum, and other important pathogenic bacteria become attenuated when they lead a saprophytic existence for some time, and regain their virulence when they are propagated within the bodies of susceptible animals.

In a later communication (March 21st, 1881) Pasteur says that he has found by experiment that when attenuated varieties of the anthrax bacillus form spores, these again reproduce the same pathogenic variety, so that cultures of each degree of attenuation can be maintained indefinitely.

On June 13th, 1881, Pasteur communicated the results of his famous experiment at Pouilly-le-Fort, near Melun. He says:

> "On the 5th of May, 1881, we inoculated, by means of a Pravez syringe, twenty-four sheep, one goat, and six cows, each animal with five drops of an attenuated culture of the anthrax bacillus. On the 17th of May we reinoculated these animals with a second virus, also attenuated, but more virulent than the first.
>
> "On the 31st of May we proceeded to make a very virulent inoculation in order to test the efficacy of the preventive inoculations made on the 5th and 17th of May. For this experiment we inoculated the vaccinated animals, and also twenty-four sheep, one goat, and four cows which had not received any previous treatment.
>
> "The very virulent virus used on the 31st of May was obtained from spores preserved in my laboratory since the 21st of March, 1877.
>
> "In order to make the experiments more comparable we inoculated alternately a vaccinated and a non-vaccinated animal. When the operation was finished all of those present were invited to reassemble on June 2d, *i.e.*, forty-eight hours after the virulent inoculation was made.
>
> "Upon the arrival of the visitors on June 2d, all were astonished at the result. The twenty-four sheep, the goat, and the six cows which had received the attenuated virus, all presented the appearance of health. On the contrary, twenty of the sheep, and the goat, which had not been vaccinated, were already dead of anthrax; two more of the non-vaccinated sheep died before the eyes of the spectators, and the last of the series expired before the end of the day. The non-vaccinated cows were not dead. We had previously proved that cows are less subject than sheep to die of anthrax. But all had an extensive œdema at the point of inoculation, behind the shoulder. Certain of these œdematous swellings increased during the following days to such dimensions that they contained several litres of liquid, deforming the animal. One of them even nearly touched the earth. The temperature of these cows was elevated 3° C. The vaccinated cows did not experience any elevation of temperature, or tumefaction, or the slightest loss of appetite. The success, therefore, was as complete for the cows as for the sheep."

The facts that infection depends to some extent upon the number of bacilli introduced, and that animals which have a certain degree of immunity, like the Algerian race of sheep, may succumb when they are inoculated with a certain quantity of virus, although they resist a smaller amount, were announced by Chauveau in his communication to the French Academy at the séance of June 28th, 1880. He says:

> "The facts which I have just presented show that the anthrax bacillus behaves in the organism of Algerian sheep, not as if it were deprived of the principles necessary for its development, but rather as if it were in a medium rendered unsuitable for its growth by the presence of substances injurious to it. In a very small number the bacilli are arrested in their development by the inhibitory influence of these substances. When they are very numerous, on the contrary, they surmount more easily this obstacle to their proliferation."

This quotation shows that Chauveau had at this early date arrived at an explanation of immunity very nearly in accord with that which is now generally accepted.

The fact that infection is influenced by the quantity of the infectious material introduced had previously been insisted upon by Davaine in his paper entitled "*Recherches sur quelques unes des conditions qui favorisent ou qui empêchent le dévelcpment de la septicémie,*" published in the *Bulletin of the Academy of Medicine,* séance of February 18th, 1879.

Davaine says:

> "A third condition relates to the quantity of bacteria introduced into the tissues. This question of quantity has been made manifest in our experiments. Not only does it differ in different species of animals, the rabbit and the dog, for example, but it varies in different animals of the same species."

In his communication to the Academy of Sciences, made on April 4th, 1881, Chauveau gives the results of his experiments in producing immunity by inoculations with very small quantities of virus. After some preliminary experiments with a larger number, five sheep were inoculated with diluted anthrax blood estimated to contain two hundred and fifty bacilli for each. All of the animals survived the inoculation after having manifested some slight febrile reaction. Six weeks later all were reinoculated with a dose which should have been fatal to an unprotected animal. One of the animals died of anthrax, the other four resisted perfectly.

On June 26th, 1882, Chauveau reported to the Academy of Sciences the results of his experiments relating to the protection of animals from anthrax infection by the method of Toussaint. By carefully conducted experiments Chauveau found that nine or ten minutes' exposure to a temperature of 54° C. killed all of the bacilli in anthrax blood, and the same result was obtained by sixteen minutes' exposure to 52° C., while at 50° C. the time required is twenty minutes. An attenuated virus suitable for protective inoculations is obtained by exposure for a somewhat shorter time, and as a result of his experiments Chauveau was led to the conclusion that for a first inoculation anthrax blood heated to 50° C. for fifteen minutes afforded a good attenuated virus. This was to be followed after an interval of ten to fifteen days by a second inoculation with a stronger virus, obtained by exposing anthrax blood to the same temperature (50° C.) for nine or ten minutes. These inoculations sufficed to protect the animals when they were subsequently inoculated with virus of full strength—blood from an animal which had recently succumbed to the disease. Chauveau says with reference to this method:

> "In one hour, with a single guinea-pig [dead of anthrax], it is easy to prepare the quantity of vaccine required to inoculate more than five hundred sheep. The inoculation is made with the point of a lancet, charged, by the method in use in my laboratory, with a very small quantity of virus. Two

or three large punctures under the skin, upon the internal surface of the ear, suffice for a successful inoculation.

"The vaccine prepared in this way should be used at once, or at least not later than the day after it has been prepared. Experience has shown me that it is then quite as harmless and quite as efficacious as Pasteur's vaccine."

In the preparation of an attenuated virus by this method Chauveau insists upon attention to the following points:

"The first rule to follow, and the principal one, is to practise the heating in such a manner that all parts of the anthrax blood are raised to the required temperature and withdrawn from it at the same instant. When the quantity of blood to be transformed to a vaccine is too great, all parts are not uniformly acted upon by the very short exposure to heat; the virulent agents in the deeper layers may, in that case, preserve all of their activity, and cause a fatal infection. To avoid this it is best to enclose the blood in little cylindrical pipettes, 1 mm. in diameter. The extremity of these pipettes is sealed, and the portion which contains the blood is immersed in a considerable quantity of water maintained at the proper temperature. At the end of the proper time they are taken from the hot bath and plunged into cold water.

"Another rule should be rigorously observed if one wishes to be sure of success. The blood should be collected under conditions which make it sure that the virulent agents introduced into the tubes all have the same vitality, the same activity, and that they are impressed in the same degree by the heating. This is the case when we take the blood from a guinea-pig just dead, after having survived from thirty-six to forty-eight hours an inoculation with very active virus. Before introducing the blood into the pipettes it should be allowed to coagulate, and the coagula should be broken and crushed in order to obtain a defibrinated blood, which is always very rich in virulent bacilli."

In a subsequent communication (February 26th, 1883), Chauveau admits that the application of this method is somewhat difficult and delicate when blood is employed, and states that it is far more satisfactory to use pure cultures, which may be attenuated in the same way. He prefers to cultivate the bacillus in a bouillon made from the flesh of a chicken, and to start his culture by adding to this bouillon a drop of blood from an animal just dead from anthrax. The culture is left for twenty hours in an incubating oven at a temperature of 43° C. During this time there is an abundant development of the bacillus, and the culture is ready to be subjected to the attenuating action of a higher temperature. This is accomplished by exposure to a temperature of 47° C. for a period of one, two, three, or four hours, according to the degree of attenuation desired. After three hours' exposure the attenuated culture no longer kills guinea-pigs. In a later communication (March 5th, 1883) Chauveau states that he has ascertained by experiment that the degree of attenuation produced by this method is maintained in subsequuent cultures made at 43° C., from the attenuated culture thus obtained.

Another method of attenuating the virulence of anthrax cultures is that described by Chauveau, in 1885. This consists in cultivating the bacillus at a temperature of 38° to 39° C., under a pressure of eight atmospheres. Cultures treated in this way killed guinea-pigs, but did not kill sheep, cattle, or horses, and constituted a suitable attenuated virus for protective inoculations in these animals. One drop was used for a sheep, and two drops for a cow or a horse, and the immunity was proved to last for a year.

Kitt, in experiments made in 1884 and 1885, found that an attenuation of the virulence of anthrax bacilli may be effected by passing them through birds, which have but little susceptibility to anthrax infection; but the results obtained were not uniform, and the method was not thought to have any great practical value. In the same paper Kitt gives an account of his experiments with Pasteur's vaccine, No. 1 and No. 2, which he obtained from the agent in Paris. These experiments led him to the conclusion that the attenuated cultures used by Pasteur are too weak. But by passing them through guinea-pigs their virulence was increased so that they served to protect cattle and sheep, although not without danger for the last-mentioned animals.

During the year 1882 Pasteur's method was extensively practised in the department of Eure-et-Loir, where anthrax was very prevalent and had been the cause of extensive losses. The results of these protective inoculations were reported to the Academy of Sciences (séance of December 18th, 1882) by Pasteur, who submitted, with some remarks, a report prepared by M. Boutet, from which we quote as follows:

> "The number of sheep vaccinated during the year has been 79,392; among these flocks the average annual loss during the past ten years was 7,237—9.01 per cent. Since the vaccinations but 518 animals have died—0.65 per cent. We must observe that this year, probably on account of the great humidity, the mortality in Eure-et-Loir has only been three per cent. The losses should therefore have been 2,382, instead of 518, without the vaccinations. In the flocks which were only partly vaccinated we had 2,308 vaccinated and 1,659 not vaccinated; the loss among the first was 8, or 0.4 per cent.; among the second the loss was 60, or 3.9 per cent. We call attention to the fact that in these flocks, in different cantons of the department, the sheep vaccinated and not vaccinated were subjected to the same conditions of soil, of lodging, of food, of temperature, and that consequently they were exposed to identical influences.
>
> "The veterinary surgeons in Eure-et-Loir have vaccinated 4,562 animals of the bovine species. Out of this number the annual loss had been 322. Since the vaccinations only 11 cows have died. That is, the annual mortality has been reduced from 7.03 per cent. to 0.24 per cent.
>
> "Some engorgements, generally not serious, having occurred after vaccinating horses, and the mortality not being great in this species, the veterinarians have thought it prudent not to vaccinate horses on a large scale. Only 524 were vaccinated; three of these died after the first vaccination."

Notwithstanding this favorable report some bacteriologists, and notably Koch, were not disposed to admit the practical value of Pasteur's anthrax inoculations. At the conclusion of an elaborate memoir published in the second volume of the "Mittheilungen" of the Imperial Board of Health of Germany (1884), Koch and his collaborators (Gaffky and Löffler) say:

> "As now a certain immunity against inoculated anthrax cannot be obtained by the method of Pasteur, as we have seen, without considerable losses, and as the immunity secured at the expense of considerable loss is only an imperfect protection against contracting anthrax in the ordinary way, we must consider the protective inoculations heretofore practised as of doubtful utility, especially when we remember that the second inoculation with a yet stronger virus causes the death of more animals which may serve to further spread the disease."

The attenuating influence of light on the anthrax bacillus and the fact that cultures attenuated in this way may be used for protective inoculations was first ascertained by Arloing (1886). Roux subsequently (1887) showed that the presence of oxygen is a necessary factor in the sterilization of cultures by exposure to sunlight. Behring, who has since been so active in the field of research to which the present volume relates, published an article in the *Centralblatt für klinische Medicin* in 1888 (September 22d) in which he attempted to explain the natural immunity of white rats against anthrax infection. His conclusions are given as follows:

> "1. The blood-serum of white rats is not a favorable medium for the anthrax bacillus."
>
> "2. The blood-serum of rats differs from that of animals susceptible to infection by its greater alkalinity."
>
> "3. By the addition of an acid to the blood-serum of rats this becomes a favorable medium for the growth of the anthrax bacillus."
>
> "4. The blood-serum of rats which are treated, during life, in such a way as to reduce the alkalinity of the blood becomes a suitable medium for the development of the anthrax bacillus."

As we have pointed out in the chapter on Natural Immunity, the true explanation of the facts ascertained in Behring's experiments is probably to be found, not in the germicidal power of the comparatively small amount of alkali present in the rat's serum, but in the fact that the germicidal proteid produced by the leucocytes is only soluble in an alkaline medium. In a paper published in the *Annals of the Pasteur Institute* (August, 1888), Roux and Chamberland have given an account of experiments made by them which establish the fact that immunity against anthrax may be established by inoculating susceptible animals with blood from an animal dead from anthrax, in which the anthrax bacilli had been killed by heat or removed by filtration (Sur l'immunité contre le charbon conférée par des substances chimiques). These experiments were commenced in 1881. The authors named say:

> "In repeating the experiments of Toussaint upon anthrax blood which had been heated, we made several observations which convinced us that it is possible to confer immunity against anthrax upon sheep by injecting under their skin anthrax blood which does not contain any living bacilli."

While immunity was produced in this way, Roux and Chamberland remark that the sheep which had received a comparatively large dose were quite sick when subsequently inoculated with a virulent culture, and the immunity acquired was less reliable than that obtained by Pasteur's method with two vaccines of different degrees of attenuation.

In an investigation made by Hankin, in the laboratory of Professor Koch at the Hygienic Institute of Berlin, the results of which are given in a preliminary account published in the *British Medical Journal* (October 12th, 1889), the important fact was ascertained that immunity may be produced in susceptible animals by inoculating them with an "albumose" isolated from anthrax cultures. Hankin gives the following account of his method of obtaining this immunizing proteid from anthrax cultures:

> "In the course of my process of preparation it is precipitated from its solution by the addition of a large bulk of absolute alcohol, and well washed in this liquid to free it from ptomaïnes; it is well known that all such substances are soluble in alcohol. It is then filtered off and dried; then it is redissolved and filtered through a Chamberland filter. A rough estimate of the percentage of albumose present in the clear solution thus obtained is made colorimetrically by means of the biuret reaction and a peptone solution of known strength."
>
> "In one experiment four rabbits (Nos. 23 to 26) were inoculated subcutaneously with virulent anthrax spores. No. 26 served as a control and died in about forty hours. The other three rabbits had the albumose solution injected into the ear-vein at the same time. Nos. 24 and 25 each had about the five-millionth of their body-weight, while No. 23 had only the ten-millionth of its body-weight of albumose. No. 25 died in less than forty-eight hours, but Nos. 23 and 24 survived. Ten days later Professor Koch kindly reinoculated these two rabbits for me with very virulent anthrax from an agar-agar culture. Their temperature has remained normal since then, and they are now alive and well a fortnight after this operation. I have also succeeded in producing immunity in mice against attenuated anthrax."

In a paper published in the Proceedings of the Royal Society in 1890, Dr. Sidney Martin has given an account of his researches relating to "The Chemical Products of the Growth of Bacillus Anthracis, and their Physiological Action." In his experiments the cultures were maintained for from ten to fifteen days, and the bacilli were then removed by filtering through a Chamberland filter. The filtrate was found to contain:

> "1. Proto-albumose, deutero-albumose, and a trace of peptone, all with the same chemical reactions as the similar bodies formed in peptic digestion. 2. An alkaloid. 3. Small quantities of leucin or tyrosin. The chief characteristic of the proto- and deutero-albumose obtained from anthrax cultures was found to be their strong alkalinity in solution. This was not removed by prolonged dialysis or by washing in alcohol, chloroform, benzene, or ether. These proteids are precipitated in an alkaline condition by saturation with NaCl (proto-albumose) or $(NH_4)2SO_4$."

The alkaloid found was soluble in water or in absolute alcohol, was strongly alkaline in solution, and readily formed salts with acids. It was slightly volatile and lost its poisonous properties to a great extent when exposed to the air for some time. A mixture of the two albumoses was toxic, and when injected into mice in small amounts caused a local subcutaneous œdema ending in recovery. Larger doses caused more extensive œdema and death. A fatal dose for a mouse weighing twenty-two grammes was 0.3 gramme. Boiling for a short time diminished the toxicity of these proteids without completely destroying it. The alkaloid produced similar symptoms when injected into mice, but more promptly and in a smaller dose—0.1 to 0.15 gramme killed a mouse weighing twenty-two grammes in two or three hours. Hankin and Westbrook have more recently (1892) made researches with reference to the proteids present in anthrax cultures. To obtain an immunizing albumose they cultivated the bacillus at 20° C. in flesh-extract solution (1:1,000) to which fibrin was added. At the end of eight days a considerable precipitate was obtained by means of ammonium sulphate. This was placed in a dialyzer in running water at 42° to 45° C.; then precipitated by alcohol and dissolved in a small quantity of water (thirty cubic centimetres)—five hundred cubic centimetres of flesh extract treated in this way gave only 0.44 gramme of albumose. Experiments on mice gave some evidence of the immunizing action of this albumose, but the results were apparently not so definite as those previously reported by Hankin. Nor are the experiments of Petermann, who followed Hankin's method (1892), more satisfactory. Arloing obtained more favorable results by using culture liquids from which the bacilli had been removed by sedimentation. A considerable precipitate was obtained when alcohol was added to the culture liquid, but it was found that this precipitate had no immunizing effect. On the contrary, there remained in solution an immunizing substance. This was obtained in a concentrated form by evaporating at 50° C. in a partial vacuum. Experiments upon lambs showed the protective power of this extract, and of the culture liquids before treatment when injected in considerable quantity.

In a paper published in the *Fortschritte der Medicin*, Wysokowicz gives a *résumé* of the results obtained in Russia in protective inoculations made up to date of publication (January, 1889). According to the author named, Professor Cenkowski, who had made himself familiar with Pasteur's method while on a visit to Paris, was the first to employ it in Russia (1883). But he found its application to be attended with some difficulties. The cultures attenuated as directed by Pasteur at 42° to 43° C. "showed a very different degree of virulence in different experiments, and their virulence was also changed by keeping." Experiments were therefore made with a view to securing a more satisfactory vaccine. In an experiment made in 1885, 1,333 sheep were inoculated; of these 21 died from the first inoculation and 4 from the second (1.86 per cent). Subsequently better results were obtained, and up to the end of 1888, 20,310 sheep had been inoculated, with an average mortality of 0.87 per cent as a result of the inoculations.

Professor Cenkowski found that greater losses occurred when the inoculations were made in midsummer or midwinter than when they were made in the spring or autumn. The losses from anthrax diminished among the flocks in which the protective inoculations were practised in proportion to the number of sheep inoculated, falling from 8.3 per cent in 1884, the year before the inoculations were commenced, to 0.13 per cent in 1888. The author of the paper states that in some parts of Russia the annual loss among the sheep from anthrax is as high as 33 per cent.

The reliability of the protective inoculations was tested by a commission, to which Wysokowicz belonged. Fifty sheep which had been inoculated from two to four months previously were infected with virulent anthrax material. Of these only one died. Later, twenty sheep which had been inoculated thirteen months before were inoculated with virulent material. Of these two died. These favorable results are ascribed by Wysokowicz to the improved method of attenuating anthrax virus adopted by Professor Cenkowski. As a first vaccine he employed a culture which was stronger than that of Pasteur, and which killed mice and caused the death of one-third of the Zieselmäuse (Spermophilus citillus) inoculated. He used as a vaccine an attenuated culture which had been carried through a series of the animals last mentioned. His vaccine, consisting of a bouillon culture from a drop of blood of the animal, was preserved by the addition of two parts of a thirty-per-cent solution of pure glycerin to one part of the culture.

For inoculating a sheep of average size he used 0.1 to 0.2 cubic centimetre of this first vaccine; for a larger animal, from 0.3 to 0.5 cubic centimetre. The second inoculation was made twelve days after the first, with a virus which killed three-fourths of the Zieselmäuse and from one-third to one-half of the rabbits inoculated with it. Numerous experiments convinced Cenkowski that no change occurred in the virulence of his different vaccines when they were carried through a series of mice or of earless marmots (Zieselmäuse).

Hess reports that the anthrax inoculations made by Chauveau's method in the Canton Bern, during the years 1886, 1887, and 1888, were not attended with any losses either from the inoculations or from subsequent attacks of anthrax among the inoculated animals (cattle?). In all, two hundred and fifty-three animals were inoculated during the three years specified.

Hutyra (1890) has reported upon anthrax inoculations by Pasteur's method, as carried out under the regulations of the Government in 1889. The number of horses inoculated was 130, 2 of which died of anthrax at a later date—not as a result of the inoculation. This gives a percentage of loss of 1.35, which is much below the usual rate without protective inoculations. Three thousand two hundred and seventy-nine cattle, belonging to 32 different estates, were inoculated. Of these 11 died from anthrax, and 2 of these as a result of the first inoculation. Deducting these 2 the loss was 0.27 per cent, whereas in former years the losses in the same herds had been from 6 to 12 per cent. Twenty-two thousand seven hundred and sixty-seven sheep were inoculated on 23 different estates. One hundred and sixty-two of these died from the first inoculation and 59 within twelve days after the second inoculation. In the course of the year 432 of the inoculated animals died from anthrax—in all a loss of 2.18 per cent. In the absence of protective inoculations the annual loss in these flocks had been about 10 per cent. It was found that lambs four months old could be inoculated with the same dose as the older sheep, and without any greater loss as a result of the operation.

The result of anthrax inoculations made in France by Pasteur's method during the twelve years ending in 1894 have been summarized by Chamberland. The veterinarians who made the inoculations were each year called upon to answer the following questions: 1. Number of animals inoculated. 2. Number of deaths from first inoculation. 3. Number of animals dying within twelve days after second inoculation. 4. Number of animals dying of anthrax within a year after protective inoculations. 5. The yearly average loss before inoculations were practised. The total number of animals inoculated during the period to which this report refers was 1,788,-

677 sheep and 200,962 cattle. The average annual loss before these protective inoculations were practised is said to have been about ten per cent for sheep and five per cent for cattle. The total mortality from this disease among inoculated animals, including that resulting from the inoculations, was 0.94 per cent for sheep and 0.34 per cent for cattle. Chamberland estimates that the total saving as a result of the inoculations practised has been 5,000,000 francs for sheep and 2,000,000 francs for cattle.

Podmolinoff gives the following summary of results obtained in 1892 and 1893 in the "government of Cherson" (Austria): Number of sheep inoculated, 67,176; loss, 294 = 0.43 per cent. Number of horses inoculated, 1,452; loss, 8. Number of cattle inoculated, 3,652; loss, 2. The conclusion is reached that Pasteur's method of inoculation affords an immunity against infection with virulent anthrax bacilli in greater amounts than could ever occur under natural conditions.

BUBONIC PLAGUE.

A number of prominent bacteriologists have been engaged in researches relating to the prevention and cure of bubonic plague by means of an antitoxic serum, obtained by the same method and in accordance with the same fundamental scientific principle as in the case of the antitoxic serum which is now so successfully employed in the treatment of diphtheria. The experiments thus far made have apparently been attended with a considerable degree of success. Professor Calmette reports that the serum of Yersin prepared at the Pasteur Institute in Paris proved to be curative in a considerable proportion of the cases treated during the recent outbreak at Oporto, and that protective inoculation conferred a temporary immunity, which, however, did not last longer than twenty days. The mortality in cases not treated by Yersin's serum was 70 per cent, in those treated with it 13 per cent.

The inoculations made by Haffkine in Bombay appear to have been quite successful. In his first experiment 8,142 persons were inoculated. Of these 18 subsequently contracted the disease and 2 died. Among 4,926 persons inoculated a single time at Dharwan, 45 were subsequently attacked and 15 died; while among 3,387 persons in whom a second inoculation was made, only 2 were attacked. Haffkine uses in his inoculations a sterilized culture of the plague bacillus. The inoculation is followed by slight fever and enlargement of the nearest lymphatic glands. All symptoms disappear at the end of two or three days.

The duration of the immunity resulting from these inoculations has not been definitely determined, although in a majority of those inoculated it appears to have afforded protection for at least five or six months. Haffkine's method of preparing his material for protective inoculations is as follows: A kilogramme of finely chopped goat's flesh is macerated in diluted hydrochloric acid, and then placed in an autoclave and heated for six hours under a pressure of three atmospheres. This is filtered, neutralized with KOH, and diluted up to three litres. The plague bacillus is grown in this medium. According to Haffkine, when the bacillus is planted upon the surface of this medium, a characteristic growth results. If undisturbed for five or six days delicate thread-like processes are seen hanging in the culture medium resembling stalactites suspended from the roof of a cavern. This growth is said to be peculiar to the plague bacillus. To make the prophylactic the bacillus is grown in a darkened room in large flasks. In India it is unnecessary to use a thermostat. Five or six crops of the stalactites are grown and shaken to the bottom of the flasks. This takes about six weeks. The culture is then sterilized in a water bath at 70° C., the time required being about three hours. A little carbolic acid or thymol is then added, and the material, after shaking to distribute the bacteria, is decanted into small bottles. It is now ready for use, and is usually injected into the subcutaneous connective tissue in doses of two cubic centimetres. A second inoculation in from fourteen to twenty days is recommended by Leumann, and after this the blood of the inoculated individual usually gives the Widal reaction.

CHICKEN CHOLERA.

Pasteur's researches with reference to the etiology of the disease known in France as *choléra des poules* first led him to the discovery that a virulent culture of a pathogenic bacterium may become "attenuated" by certain agencies, and that immunity may be conferred upon susceptible animals by inoculating them with such attenuated culture. We now know that his microbe of fowl cholera is a widely distributed bacillus, which is frequently encountered in putrefying material, and that it is also extremely fatal to pigeons, pheasants, sparrows, rabbits, and mice. Also that the same or nearly allied species may produce an infectious disease of swine (*Schweineseuche*), of cattle (*Rinderseuche*), and of deer (*Wildseuche*).

Subcutaneous injection of a minute quantity of a virulent culture usually kills chickens within forty-eight hours. Some time before death the fowl falls into a somnolent condition, and, with drooping wings and ruffled feathers, remains standing in one place until it dies. Infection may also occur from the ingestion of food moistened with a culture of the bacillus or soiled with the discharges from the bowels of other infected fowls. At the autopsy the mucous membrane of the small intestine is found to be inflamed and studded with small hemorrhagic foci, as are also the serous membranes; the spleen is notably enlarged. The bacilli are found in great numbers in the blood, in the various organs, and in the contents of the intestine. In rabbits death commonly occurs in from sixteen to twenty hours, and is often preceded by convulsions. The temperature is elevated at first, but shortly before death it is reduced below the normal. The post-mortem appearances are: swelling of the spleen and lymphatic glands; ecchymoses or diffuse hemorrhagic infiltrations of the mucous membranes of the digestive and respiratory passages, and in the muscles; and at the point of inoculation a slight amount of inflammatory œdema. The bacilli are found in considerable numbers in the blood within the vessels, or in that which has escaped into the tissues by the rupture of small veins. They are not, however, so numerous as in some other forms of septicæmia—*e.g.*, anthrax, mouse septicæmia—when an examination is made immediately after death; later, the number may be greatly increased as a result of post-mortem multiplication within the vessels. The rabbit is so extremely susceptible to infection by this bacillus that inoculation in the cornea by a slight superficial wound usually gives rise to general infection and death. This animal may also be infected by the ingestion of food contaminated with a culture of the bacillus. It is by this means that Pasteur proposed to destroy the rabbits in Australia, which have multiplied in that country to such an extent as to constitute a veritable pest. Both in fowls and in rabbits the disease may, under certain circumstances, run a more protracted course—*e.g.*, when they are inoculated with a small quantity of an attenuated culture. In less susceptible animals—guinea-pigs, sheep, dogs, horses—a local abscess, without general infection, may result from the subcutaneous injection of the bacillus; but these animals are not entirely immune. In the infectious maladies of swine, cattle, deer, and other large animals, to which reference has been made, and which are believed to be due to the same bacillus, the symptoms and pathological appearances do not entirely correspond with those in the rabbit or the fowl; but the bacillus as obtained from the blood of such animals corresponds in its morphological and biological characters with Pasteur's microbe of fowl cholera, and Koch's bacillus of rabbit septicæmia, and pure cultures from the various sources mentioned are equally fatal to rabbits and to fowls. In the larger animals pulmonary and intestinal lesions are developed, and in swine a diffused red color of the skin, similar to that observed in the disease known in Germany as *Schweinerothlauf* (Fr. *rouget*) is sometimes seen.

According to Baumgarten, bacilli from *Wildseuche* or from *Rinderseuche* inoculated into swine give rise to fatal *Schweineseuche*, and bacilli from any of these forms of disease, when inoculated into pigeons, produce characteristic fowl cholera; but the bacillus as obtained from *Schweinesuche* or *Wildseuche* is not fatal to chickens, and the bacillus from *Schweineseuche* is fatal to guinea-pigs, which have but slight susceptibility to the bacillus of rabbit septicæmia. Notwithstanding these differences, he agrees with Hueppe in the view that the bacilli from the various sources mentioned are specifically identical; although evidently, if this view is adopted, we must admit that varieties exist which differ somewhat in their pathogenic power.

In this volume this bacillus is described under the name *Bacillus septicæmiæ hæmorrhagicæ*, first proposed for it by Hueppe. In the present chapter we shall give an account of the experimental evidence relating to protective inoculations in various animals, with the different varieties of the bacillus in question which have been encountered.

It seems probable that the same bacillus was the cause of the fatal form of septicæmia studied by Davaine, which resulted from the inoculation of susceptible animals with putrefying blood. These experiments by the distinguished French physician constitute an important part of the pioneer work in this field of research. They were commenced in 1868, and are published in the *Bulletin of the Academy of Medicine* (séance of Feburary 18th, 1879).

Davaine, in the paper referred to, calls attention to the fact, developed by his experiments, that there is a great difference in the resisting power of different animals to the form of septicæmia which had been the subject of his investigations. Thus the rabbit succumbed when inoculated with a millionth part of a drop of blood, while guinea-pigs and dogs remained unaffected by such small doses. With reference to the specific cause of the form of septicæmia studied by him, Davaine says:

> "The virus is one of the bacteria of putrefaction. I say 'one of the bacteria,' because there is reason to believe that there are among these minute organisms numerous species which do not all develop at the same time when they are present in various media."

Davaine also discovered the fact that infection depends, within certain limits, upon the quantity of bacteria introduced into the tissues. He says:

> "This question of quantity was manifest in our experiments. Not only did it vary in different species, the rabbit and the dog, for example, but it may vary in the same species."

The identity of "Davaine's septicæmia" with Pasteur's *choléra des poules* is made still more probable by the experimental evidence offered by Toussaint in a communication to the French Academy of Sciences, made by M. Bouley at the séance of July 25th, 1881. In this communication Toussaint says:

> "Three years ago, July 8th, 1878, I had the honor to present to the Academy an account of a malady due to microbes, which I identified with that studied by Davaine in 1864 and 1865, and which he differentiated from anthrax, for which it had been mistaken by Leplat and Jaillard.
>
> "In the month of December, 1878, I made acquaintance with fowl cholera, and already, in my thoughts, I identified this disease with that which I had observed in my experiments made early in the year. The microbes of the two diseases resembled each other perfectly and behaved the same when inoculated in rabbits. I had, even in 1879, sent to M. Bouley two notes, in which I called attention to the analogies which exist between the parasites of the two diseases and the lesions which they determine, not only in the rabbit but also in pigeons and fowls.
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> "The experiments of the same kind made at the end of 1879 and in 1880 caused me to insert the note published on page 301, vol. xci., of the *Comptes-rendus*, under the title of 'Identity of Acute Experimental Septicæmia and Fowl Cholera.' I gave a *résumé* in this note of five series of experiments which had demonstrated to me that inoculations of the microbe of septicæmia give rise to the manifestations of fowl cholera. These results have recently been confirmed by additional facts."

Toussaint closes his paper by some remarks upon the origin of epidemics of fowl cholera, which we quote because we believe that the additions made to our knowledge of the microbe which causes this disease give support to the views advanced by him in 1881:

> "The causes which determine epidemics of fowl cholera are yet unknown. It has been supposed that putrefactive substances may give rise to them, and this has led to the recommendation of cleanliness and disinfection for their prevention. The microbe which kills the first fowl in an epidemic certainly came from some anterior generation which had killed others. But how was it perpetuated? Do not the facts which demonstrate the development of septicæmia from material undergoing putrefaction throw some light on the question of etiology? Is it not probable that the fowls find the conditions of infection with cholera in the presence of organic matter undergoing putrefaction, which may serve as a culture medium for the germs of septicæmia which are in suspension in the air together with the ordinary germs of putrefaction?"

Pasteur's first communication relating to the etiology of fowl cholera was made to the French Academy at the séance of February 9th, 1880. In this communication he calls attention to the fact that when fowls are fed with bread or meat soiled with a small quantity of a culture of the microbe of fowl cholera they become infected and their discharges contain the bacillus in large numbers, a fact which readily accounts for the spread of the disease in a poultry yard when a case occurs.

In the same communication Pasteur records his observation that "by a certain change in the method of cultivation the infectious microbe may be caused to have a diminished virulence." Also the fact that fowls inoculated with this "attenuated" virus recover and are subsequently immune against infection by the most virulent microbes. In concluding this communication Pasteur says:

> "It appears to be superfluous to point out the principal result of the facts which I have had the honor to present to the Academy. There are two, however, which it may be useful to mention. These are, first, the hope of obtaining artificial cultures of all kinds of virus; second, the idea of seeking for virus vaccines of the virulent maladies which have devastated so often, and still devastate, the human race, and are such a scourge to that branch of agriculture which relates to the breeding of domestic animals."

In his communication of October 26th, 1880, Pasteur gives his reasons for concluding that attenuation of virulence is due to the action upon the microbe of atmospheric oxygen. He infers this from the fact, demonstrated by experiment, that when cultures are placed in hermetically sealed tubes, from which the oxygen present is soon exhausted by the growth of the microbe, they do not become attenuated in virulence; whereas cultures which are freely exposed to the air gradually become attenuated. Pasteur sees in this an important fact bearing upon the explanation of the natural extinction of epidemics. He says:

> "May we not suppose, then, that it is to this influence that we must attribute, in the present as in the past, the limitation of great epidemics?"

In his communication to the French Academy, made on February 28th, 1881, Pasteur treats of the attenuation of virulence by the method above referred to and by the method of Toussaint, and also of the reestablishment of the virulence of attenuated cultures. He says:

> "The secret of the return to virulence rests solely, at present, upon successive cultures in the bodies of certain animals."

Thus he had found by experiment that the anthrax bacillus might be so attenuated that it was harmless for grown guinea-pigs, or even for guinea-pigs a month or a week old, but it would still kill guinea-pigs just born—a day old. By inoculating an older pig with the blood of this one, and so on, the virulence was gradually augmented, until finally a virus might be obtained which would kill adult animals, and even sheep. In the same way the attenuated microbe of fowl cholera could be restored to virulence by first inoculating small birds, such as sparrows or canaries.

Applying these facts, demonstrated by his experiments, to the explanation of the origin of epidemics, Pasteur says:

> "I finished my communication on October 26th by calling attention to the attenuation of viruses by exposure to the air as being probably one of the factors in the extinction of great epidemics. The facts presented in this paper, in their turn, may serve to explain the so-called 'spontaneous development' of these scourges.
>
> "An epidemic which has been extinguished by the attenuation of its virus may be reborn by the reinforcement of this virus under certain influences. The accounts which I have read of the spontaneous appearance of the plague appear to me to offer examples of this; for example, the plague at Benghazi, in 1856–58, the outbreak of which could not be traced. The plague is a virulent malady which belongs to certain countries. In all of these countries its attenuated virus ought to exist, ready to resume its active form when conditions as to climate, famine, and distress again occur. There are other virulent maladies which appear 'spontaneously' in all countries; such as camp typhoid. Without doubt the germs of the microbes which cause these last-mentioned maladies are everywhere distributed. Man carries them upon him or in his intestinal canal without great damage, but ready to become dangerous, when, owing to constipation or to successive development upon the surface of wounds, in bodies enfeebled or otherwise, their virulency is progressively reinforced."

We believe that the more complete our knowledge relating to the origin and extinction of epidemics, of the kind referred to by Pasteur, becomes, the more apparent will be the value of his inductions and the clearness of his scientific foresight.

Toussaint, on July 25th, 1881, reported the results of his experiments upon protecting fowls by a "new method of vaccination." This consisted in inoculating them with the blood of a rabbit which had recently died from septicæmia produced by the same microbe. As a result of such inoculations the fowls had slight local lesions at the point of inoculation, and soon recovered. They were subsequently found to be immune. Cultures from the blood of a septicæmic rabbit were found to act in the same way. When the culture had been passed through a pigeon, and had then killed a fowl, according to Toussaint, it preserved its virulence when subsequently passed through the rabbit.

Salmon, in the "Report of the Commissioner of Agriculture" for 1881 and 1882, gives an account of his experiments in producing immunity by the use of a diluted virus. He says:

> "The experiments of Chauveau, taken with my own, indicate that this method is capable of generalization to the same extent as that discovered by Pasteur: while the ease and quickness with which the vaccine is prepared, the certainty of effects, the economy of material, and the more perfect protection are points which would appear to make it decidedly superior. Wherever the cholera of fowls is raging a standard cultivation may be made and the vaccine obtained within twenty-four hours; a single drop of such a cultivation will vaccinate ten, twenty, or even forty thousand fowls, and within three weeks of the commencement of work the most susceptible of our

fowls are insusceptible to inoculation with the strongest virus. And this, without any sickness, or even local necroses, which Pasteur describes as following vaccinations with his attenuated virus."

In discussing the practical value of this method Salmon estimates the cost as trifling—"not more than half a day's time of one man for one hundred fowls, even if three inoculations were made."

In a paper on protective inoculations against fowl cholera, by Kitt, in the *Deutsche Zeitschrift für Thiermedicin* (December 20th, 1886), the conclusion is reached that these inoculations undoubtedly protect the fowls from infection either in the natural way or by inoculations with virulent material. But Kitt doubts the practical utility of the method for the arrest of epidemics of this disease in the poultry yard; and, as we think with justice, prefers to depend upon cleanliness, disinfection, and prompt removal of infected fowls. As he points out, a considerable time is required to produce complete immunity, and two inoculations are often insufficient. Pasteur had previously reported that a third inoculation is usually required. But the infection spreads so rapidly when an epidemic is developed in a poultry yard that a large proportion of the fowls would be likely to perish before the protective inoculations could be carried out. Another objection is that when inoculated in the breast muscle the value of the fowl for the table is reduced, and when inoculated in the wing an unpleasant-looking scab is left at the point of inoculation. The cost in material and time required to carry out the three successive inoculations is also an objection to the practical application of the method. Moreover, the excreta of the inoculated fowls contain the pathogenic microbe, and it would evidently be unwise to practise inoculations in poultry yards not already infected. Kitt states also that he has always succeeded in stamping out the disease very promptly by the other measures referred to—disinfection, cleanliness, separation of all fowls which show any indications of being infected.

In a more recent paper (1893) Kitt reports his success in conferring immunity upon fowls by a new method, which is, however, rather of scientific interest than of practical value. He first experimented to see whether the blood serum or tissue juices of immune fowls would give immunity against cholera to other fowls, and obtained a successful result. He was not, however, able to produce immunity in pigeons or in rabbits by the same method. He next undertook to determine whether the immunizing substance was present in the eggs of fowls which had an immunity as a result of protective inoculations. The albumen and yolk of the egg, in doses of five to ten cubic centimètres, was injected into the breast of fowls, and at the end of ten days a second inoculation of the same kind was made. Six days after the second inoculation the fowls (five) and a control hen were inoculated with virulent blood from a pigeon, and at the same time fed with the chopped-up flesh and liver of a pigeon just dead from fowl cholera. The control hen died on the following day from typical cholera, the others remained in perfect health.

CHOLERA.

The spirillum discovered by Koch in 1884 is now generally recognized as the specific cause of Asiatic cholera. But recent researches indicate that there are numerous pathogenic varieties of this spirillum, and show that either an attenuated cholera spirillum or a closely allied saprophyte is not infrequently found in the water of rivers in various parts of Europe. As this spirillum is found in the intestine of cholera patients, and not in the blood, it is evident that its pathogenic action depends upon the chemical products developed during its growth, and this inference is fully justified by the results of experiments upon the lower animals. These chemical products have been studied by Brieger, Pfeiffer, Scholl, Gamaleía, Westbrook, and others.

Brieger (1887) succeeded in isolating several toxic ptomaïnes from cultures of the cholera spirillum, some of which had previously been obtained from other sources—cadaverin, putrescin, creatinin, methylguanidin. In addition to these he obtained two toxic substances not previously known. One of these is a diamine, resembling trimethyldiamine; it gave rise to cramps and muscular tremor in inoculated animals. The other poison reduced the frequency of the heart's action and the temperature of the body in the animals subjected to experiment. In more recent researches made by Brieger and Fränkel (1890), a toxalbumin was obtained from cholera cultures which, when injected subcutaneously into guinea-pigs, caused their death in two or three days, but had no effect upon rabbits.

Pfeiffer has more recently (1892) published his extended researches relating to the cholera poison. He finds that recent aërobic cultures of the cholera spirillum contain a specific toxic substance which is fatal to guinea-pigs in extremely small doses. This substance stands in close relation to the bacterial cells, and is perhaps an integral part of the same. The spirilla may be killed by chloroform, thymol, or by desiccation, without apparent injury to the toxic potency of this substance. It is destroyed, however, by absolute alcohol, by concentrated solutions of neutral salts, and by the boiling temperature, and secondary toxic products are formed which have a similar pathogenic action but are from ten to twenty times less potent. Similar toxic products were obtained by Pfeiffer from cultures of the Finkler-Prior spirillum and from Spirillum Metchnikovi.

Scholl (1890) took advantage of the fact, previously demonstrated by Hueppe, that cultures of the cholera spirillum in egg albumen, in the absence of oxygen, are more toxic than ordinary bouillon cultures. Cultures were made by Hueppe's method in hen's eggs. No poisonous ptomaïnes were found, but two toxic albuminous substances were obtained. The albuminous liquid from the egg cultures was dropped into ten times its volume of absolute alcohol, which caused a white precipitate, a portion of which sank to the bottom while another portion floated on the surface. The portion which floated was easily dissolved in a very dilute solution of potash and could be precipitated from this solution by the careful addition of acetic acid, but dissolved in an excess of this acid. It dissolved also in a seven-per-cent salt solution, but was precipitated by a saturated solution. It gave the biuret and xanthoprotein reaction. This substance proved to be very poisonous. It killed guinea-pigs within twenty minutes when a few cubic centimetres of the alkaline solution—potash—were injected into the cavity of the abdomen. Scholl calls this substance cholera-toxo-globulin. The precipitate which fell to the bottom of the receptacle was washed with alcohol, then digested with water for twenty minutes at 40° C. Very little was apparently dissolved out by this procedure, but this little proved to be very toxic. In from one to three minutes after the injection of a few cubic centimetres of the solution into the peritoneal cavity of a guinea-pig the animal died. This aqueous solution gave the biuret and xanthoprotein reaction; it was precipitated by mercuric chlorid, nitrate of mercury, and tannin, but not by a saturated solution of ammonium sulphate or acetic acid. This substance Scholl calls cholera-toxo-pepton. The toxic action of these substances is destroyed by a temperature of 100° C., maintained for half an hour, or by 40° to 45° C., maintained for twenty-four hours. But at ordinary temperatures they retain their toxic action for several weeks.

Gruber (1892) has also obtained a toxic albuminous precipitate by allowing egg cultures to fall into alcohol, drying the precipitate, and then extracting it with water.

Gamaleía (1893) has obtained a toxin which produces the typical phenomena of cholera, which, according to him, is closely associated with the bacteria cells, but can be extracted by a soda solution or by heating to 55° to 60° C. The conclusion is reached that it is a nucleo-albumin analogous to the toxalbumins of tetanus and of diphtheria. It is precipitated by alcohol, acids, and by magnesium sulphate.

Finally, Westbrook, in a still more recent research (1894), arrives at the conclusion that the cholera spirillum produces various toxic proteids which in small amounts produce immunity in susceptible animals, and the production of which depends to a certain extent upon the culture medium; or that its toxin is a substance of constant chemical composition which is mixed with various albuminous substances, either contained in the culture medium or developed in the culture. Duclaux is of the opinion that the last supposition is correct, and that the so-called toxalbumins are not bodies of definite chemical composition, but mixtures of toxins and albuminous substances.

Experiments made upon the lower animals show that the introduction of these cholera toxins into the body of a susceptible animal, either with or without the living cholera spirillum, results in the establishing of a certain degree of immunity against the toxic action of cholera cultures. And there is good reason to believe that a non-fatal attack of cholera in man gives the individual a relative immunity from subsequent attacks, for some time at least. This has led to extended experiments with reference to the possibility of producing a similar immunity in man by means of protective inoculations. The experiments bearing upon this point which have been made upon the lower animals will first engage our attention.

Hueppe (1887) first demonstrated the fact that injection of a small amount of a cholera culture into the peritoneal cavity of a guinea-pig is fatal to these animals.

In the following year (1888) Gamaleía reported his success in infecting guinea-pigs by subcutaneous injections of blood from an infected pigeon. He found that by successive inoculations in pigeons a considerable increase in virulence is established; and that while guinea-pigs were not fatally infected by subcutaneous inoculations with ordinary cultures, they invariably died when inoculated with the more virulent culture in the blood of an infected pigeon. Also, that when guinea-pigs were inoculated with ordinary cultures, or with cultures sterilized by heat, they were subsequently immune, and resisted inoculations with the most virulent material. In the same year the author referred to announced the discovery of a spirillum which closely resembles the cholera spirillum—his "Vibrio Metchnikovi." This was obtained from the intestinal contents of fowls suffering from a fatal infectious malady (in Odessa). According to Gamaleía, chickens and pigeons which have survived an inoculation with a culture of this spirillum are subsequently immune against the pathogenic action of the cholera spirillum, and *vice versa*. In subsequent communications Gamaleía reported that sterilized cultures of his "Vibrio Metchnikovi" (sterilized by heat at 120° C.) were very pathogenic for rabbits, fowls, pigeons, and even for dogs and sheep. The rabbit proved to be the most susceptible animal, and succumbed to doses of four cubic centimetres in from twelve to twenty hours. Doses of one cubic centimetre per one hundred grammes of body weight caused a temporary indisposition followed by immunity. Pigeons were made immune by larger doses.

The researches of Pfeiffer (1889) confirmed those of Gamaleía as to the fact that pigeons and guinea-pigs could be made immune against Vibrio Metchnikovi by the injection of sterilized cultures. But guinea-pigs which had been immunized against this pathogenic spirillum succumbed to cholera infection; and, on the other hand, animals which had been treated in various ways with a cholera culture died without exception when infected with Vibrio Metchnikovi. The conclusion is therefore reached that the two pathogenic spirilla are distinct species, although very similar in many respects.

Brieger and Wassermann (1892) have reported the results of experiments with the cholera spirillum cultivated in thymus bouillon. After twenty-four hours' development in this medium the cultures were sterilized by heat (55° C. for fifteen minutes) and placed in an ice-chest for twenty-four hours. Four cubic centimetres of this fluid injected daily for four days into the peritoneal cavity of a guinea-pig made it immune to the cholera spirillum in doses three times as large as were required to kill an animal not so treated. This immunity lasted for two months. Fedoroff (1892) obtained similar results by the subcutaneous injection of sterilized cultures in doses of one cubic centimetre, in guinea-pigs. His cultures in thymus bouillon were kept for from seven to ten days at 37° C., then sterilized by heating for fifteen minutes at 65° C., then allowed to stand in a dark room for twenty-four hours, and finally mixed with an equal volume of glycerin.

Ketscher (1892) has obtained evidence that the immunizing substance in animals which have received protective inoculations is contained in the milk of females thus treated. Three goats received subcutaneous inoculations of virulent cholera cultures, and also injections into a vein and into the peritoneal cavity. The milk of these goats was injected into the peritoneal cavity of rabbits; these proved to be immune when subsequently lethal doses of a virulent cholera culture were injected into the peritoneal cavity.

According to Gamaleía (1892), dogs are very susceptible to infection with cholera spirilla, and present symptoms closely resembling those of cholera in man. They may also be easily immunized against the pathogenic action of cholera cultures.

Gruber and Wiener (1892) have also found that susceptible animals are easily immunized against cholera infection either by inoculation with small doses, with attenuated cultures, or with larger quantities of sterilized cultures. Haffkine (1892) also reports his success in immunizing guinea-pigs and pigeons.

Pawlowsky (1893) claims to have obtained from the blood of animals having an acquired immunity against cholera an antitoxin in the form of an amorphous powder; and Lazarus (1892) reports that the blood of man, after recovery from an attack of cholera, has the property of protecting guinea-pigs from fatal infection when injected, in very small amount, into the peritoneal cavity. Issaeff (1894) in an extended series of experiments was not able entirely to confirm the results reported by Lazarus. In a summary of results obtained in his own experiments he says:

> "1. The intraperitoneal or subcutaneous injection of blood serum from normal individuals [that is, persons who have not suffered an attack of cholera], and also of various acids, alkalies, and neutral liquids, gives to guinea-pigs a certain resistance against intraperitoneal cholera infection. This resistance, however, is feeble and temporary, and cannot be considered as identical with the true immunity which results from vaccination with the products of the cholera bacteria.
>
> "2. Guinea-pigs vaccinated against cholera have no immunity against the toxins of the cholera vibrio, notwithstanding their high degree of insusceptibility to infection with cultures containing the living vibrio. The blood of immunized guinea-pigs does not possess antitoxic properties. The maximum dose of cholera toxins which immune guinea-pigs can withstand is not greater than that which control animals withstand.
>
> "3. The blood of guinea-pigs carefully immunized against cholera possesses specific and very pronounced immunizing, and, in a certain sense, curative powers.
>
> "4. The blood of cholera convalescents possesses similar specific and curative powers. This property is first developed about the end of the third week after the attack, and disappears completely at the end of two or three months."

In a series of experiments made by Pfeiffer and Issaeff the results obtained, as stated by Pfeiffer in a subsequent communication, were as follows:

> "In my research with Issaeff 'upon the explanation of cholera immunity' I proved that the serum of animals which have an active acquired immunity against cholera only has a specific action upon this particular species of vibrio, and as regards other species of bacteria does not differ in its action from the blood serum of normal animals. We also showed that this specific influence in respect to the intraperitoneal cholera infection of guinea-pigs was due exclusively to bactericidal processes which in some way were induced by the serum of immune animals."

The view of Pfeiffer, founded upon his experimental results, is that the destruction of the living cholera spirilla, which quickly takes place in the peritoneal cavity of the guinea-pig, when at the same time a minute quantity of serum from an immune animal is introduced, is not directly due to the bactericidal action of this serum, but that in some way it gives rise to a specific bactericidal action in the exudate which is found in the peritoneal cavity as a result of such injections. His experiments also lead him to the conclusion that this is accomplished quite independently of phagocytosis.

The brief review of experimental researches relating to cholera immunity which we have made shows that, while there is a general agreement as to the possibility of producing immunity in susceptible animals, there is considerable difference of opinion as to the true explanation of this immunity. The supposition that it is due to an antitoxin which has the power of neutralizing the toxic products of the cholera spirillum does not receive any support from the most recent investigations—those of Pfeiffer and Issaeff—which, on the contrary, seem to establish the fact that this immunity depends upon an increased bactericidal activity of the blood serum of immune animals. A very curious fact developed by the researches of the bacteriologists last named is that—

> "The cholera serum which in the peritoneal cavity of guinea-pigs acted only upon the cholera bacteria, and behaved toward other vibrios exactly like the serum of normal animals, in a test tube killed all four species of vibrios with equal rapidity."

Unfortunately the evidence relating to the value of protective inoculations in man, although supported by the evidence already referred to as regards the lower animals, is, to a considerable extent, unsatisfactory, owing to the difficulty of applying scientific methods to experiments of this kind. The evidence, however, is in favor of the view that a certain degree of protection is afforded by the subcutaneous injection of cholera cultures. Such protective inoculations could not be expected to confer an absolute immunity, inasmuch as the immunity resulting from a single attack has only a relative value, and is probably not of long duration.

We quote from Shakespeare's "Report on Cholera in Europe and India, 1890," the following paragraphs relating to immunity as a result of an attack of cholera:

"IMMUNITY AFTER AN ATTACK OF CHOLERA—EXPERIENCES IN FRANCE, 1884.

"The Academy of Medicine of Paris directed a circular letter of questions concerning cholera to the physicians of the localities infected by that disease in 1884, and in group L of general observations in that questonario is found the following: 'Have there been observed recurrences among the people attacked, either in a former epidemic or in the present one? Give the results of this recurrence.' In response to their questions the Academy received 184 communications, but the committee appointed to analyze them eliminated 79; for various reasons given only 104 were used for analysis. Of this number only 8 bore upon the particular question above mentioned, and it is reasonable to assume that the other 96 observers said nothing concerning this point because they had observed nothing bearing upon it. The results of this analysis may be stated as follows:

"From Castelnaudary, with a population of 10,000, we learn that there were 54 cases and 18 deaths from cholera, among which there was 1 recurrence; from Aix, with 20,257, number of cases unknown, deaths, 117, among these 2 recurrences were observed, at intervals of ten and forty days; from Beseges, with 11,400 inhabitants, we learn of 124 cases and 40 deaths, among which were 2 recurrences; from Cette, with 35,000, the number of cases is not mentioned, but we learn that there were 92 deaths and 1 recurrence; from Nantes, with 124,300 inhabitants we learn of 251 cases and 112 deaths, with 1 recurrence; from Perpignan, with 25,000 inhabitants, we hear of 325 cases and 225 deaths, and receive the indefinite statement that there were some fatal recurrences; from Pignans, population not stated, we learn of 22 attacks and 12 deaths, with 1 recurrence; from Cadenet, with a population of 26,000, we are not informed of the number of cases, but learned that there were 20 deaths and 2 recurrences."

"IMMUNITY AFTER AN ATTACK OF CHOLERA—EXPERIENCE IN SPAIN, 1885.

"While examining cholera in Spain, the writer prepared a circular containing a series of twenty-five questions relating especially to the nature, etiology, and prophylaxis of cholera, one of which requested the physician to state whether or not, in his own personal experience, he had observed a second or a third attack of cholera during the same epidemic, and in case of a positive reply to detail the symptoms and all the circumstances surrounding it. This circular-letter was addressed to some twenty-five hundred Spanish physicians, located in the various cities, towns, and villages in that kingdom which had suffered from the epidemic. Among the large number of replies there were only eight in which a second attack was reported, and from an examination of the details of these there was no doubt left in our mind that six were not genuine second attacks after a complete recovery, but were in reality relapses due to imprudences of diet or otherwise before convalescence and complete recovery had been established. Two of the eight cases, from the details of the reports given, may have been genuine recurrent attacks of Asiatic cholera, or may have been simply seizures of cholera morbus (cholera nostras). It is well known that after an attack of Asiatic cholera the digestive apparatus is left in a damaged condition, and disorders of the intestines continue for a long time. The habits of life and the imprudences so common to the class of people most frequently suffering from Asiatic cholera in that country are such as to render them more than usually liable to suffer attacks of cholera nostras. As having an important bearing upon this suggestion, the writer made an analysis of the vital statistics of Spain, covering the five years previous to 1885, for the purpose of learning the extent of prevalence of cholera nostras among that population, and the result of the inquiry shows that the number of deaths attributed to that disease averaged per year sixteen per every million inhabitants."

Dr. Ferrán, who practised inoculations on an extensive scale during the epidemic of 1885, in Spain, gives the following account of his method of performing these inoculations:

"1. The cholera vaccine is nothing more than a pure culture, in bouillon, of the comma bacillus. Its easy and long preservation (four to five days) allows of its transportability to great distances. taking care always to keep the flask which contains the material upright.

"2. Heat and cold do not interfere with its preservation if the vaccine is to be used in a short time. It should not, however, be kept out of doors during the warm season.

"3. The vaccine should be kept in flasks of the model of Ferrán, with a flat bottom and a short neck. The stopper, which is of rubber, fits perfectly, and is penetrated by two glass tubes. One, straight and short, which does not extend below the inferior surface of the stopper. and which does not project above more than some two centimetres, is plugged with a small quantity of sterilized cotton and a superficial covering of wax. The other glass tube is longer, and extends on the lower side as far as the bottom of the flask, while its superior end is curved, and terminates in a capillary extremity, the tip of which is closed with wax.

"4. When the vaccine is to be used it is necessary to make two principal preparations for the operation. A small syringe for the hypodermic injection, and a small vessel into which it is necessary to empty the fluid from the flask, are required. The syringe should have metallic pistons and mountings, without mastic of any kind and without rubber. Its capacity should be one cubic centimetre, its needle thicker and shorter than that of ordinary use. Before beginning the vaccination the syringe must be filled two or three times with boiling water, which is aspirated and expelled through the needle. This is called sterilizing the instrument, and by this means the extraneous germs are destroyed which might be contained in it, in order to avoid the production of phlegmons and abscesses. The trouble in taking this precaution will be little. Acting thus, one may perform thousands of injections without fear of any accident. It is suggested that it is a bad custom to pass the needle through a flame in order to sterilize it, because this mode of procedure draws the temper. Another precaution that must be taken relates to the examination of the syringe before using it, in order to be well assured that the piston acts perfectly and that not a single drop of the liquid escapes by a leak in the cannula. This latter defect is sufficient to reject the instrument. If the syringe aspires air because the leather washer, which is placed at the end of the glass tube in order to facilitate its adaptation, is dry, or the piston is in the same condition, it is necessary to delay a little while in order to take the syringe apart and soak it in warm water. It is convenient to keep several syringes for use, with a sufficient number of needles, when many inoculations are to be performed.

"5. The small receptacle into which the vaccine is poured in order that the syringe may be filled readily is a capsule, a cup, or some similar vessel. Before use, it should be washed and dried with extreme care, and immediately before using passed through an alcohol or Bunsen flame, in order to sterilize it.

"6. All these preparations having been made, the drop of wax which closes the capillary extremity of the long tube of the flask is removed, and at the same time also the wax covering of the cotton stopper of the short tube, but by no means must this cotton stopper be removed; a rubber tube, or the extremity of a small Richardson spray apparatus, is adjusted to the short tube. The capillary extremity of the long tube is now slightly warmed in order to soften somewhat the wax which may have been drawn into its lumen by capillarity, and air is forced into the flask, either by blowing into the rubber tube or by working the Richardson atomizer; the air injected by pressure upon the vaccine fluid forces the latter out through the long tube with the capillary extremity, and it is collected in the cup or small sterilized vessel. This latter is then covered with white paper, which has been scorched in the flame, or with a sterilized glass plate; as often as the syringe is filled this cover will be removed and again immediately afterward replaced.

"7. Never should the rubber stopper which closes the flask, or the cotton which plugs the short straight tube, be removed, because otherwise the germs of the external air might enter and contaminate the culture, and in this way give place to local and general accidents among the inoculated. Whenever, through the movements of transportation, the cotton plug in the short glass tube has become so wet as to impede the passage of the air which is to be forced into the flask in the act of expelling the vaccine from it, it may be removed with the point of a needle and rapidly substituted by another plug of surgical cotton which has been carbonized or salicylized. If this proceeds with cleanness and promptness, there is no danger in doing it. When the cotton, although wet, does not impede the injection of the air, it is better not to change it.

"8. After terminating the vaccination, again the capillary extremity of the curved tube is passed through the flame until the small quantity of liquid remaining in it is evaporated; it is then stopped a second time with a small drop of wax; and from the other glass tube the rubber tube which has been employed for forcing in the air is removed and another thin layer of wax is placed over the cotton plug.

"9. If in the smaller vessel or cup any of the vaccine fluid remains after the vaccination of all persons present, it is boiled, and in this manner the culture is killed, for it should not be used in another operation, because atmospheric germs might become mixed with it.

"10. The technique for the practice of the inoculation is the same as for all hypodermic injections. The most convenient region is that of the brachial triceps.

"11. The dose is one cubic centimetre—or the contents of a syringe—into each arm, for individuals of all ages and conditions.

"12. Five days having elapsed, revaccinations may be performed by following the same instructions."

Shakespeare, who was sent by the United States Government to Spain to investigate the results of these inoculations, reports as follows:

"And now with respect to the human inoculations: The most of these inoculations were performed in villages in the province of Valencia. The number of persons inoculated considerably exceeds thirty thousand. Much has been both said and written in Spain, France, and England concerning the results of these inoculations. The results which have been published have appeared to very strongly back up the claim of Dr. Ferrán that choleraic inoculation has the power of protecting the individual against an attack of cholera, and that the extensive practice of this inoculation among villages already invaded by the epidemic is a powerful and at the same time harmless means of bringing the epidemic to an end. This being the case, for those who were unwilling to accept the deductions to be made from the published statistics the only way of escaping their force seemed to be by an attack upon their validity.

"The statistics of the anti-choleraic inoculations have been widely attacked. The first public onslaught upon these statistics of which the world, outside of Spain, had much knowledge was made in the report of the French Commission, with Dr. Brouardel at its head, which was presented to the Minister of Commerce after the return of that Commission from Spain in the summer of 1885. It is charged in that report that the results of the statistics therein reproduced are assailable on account of having been collected by physicians who were partisan supporters of Dr. Ferrán, and that they neither possessed any adequate official character nor did they possess sufficient details. As far as I can learn, the general impression entertained throughout the world of the value of inoculation statistics is based, in the main, upon this report of the French Commission.

"The statement of that Commission that the statistics which they had been able to obtain of the preventive inoculations of Ferrán were to a considerable degree void of any official character may be true, and perhaps it is also true that they emanated from the partisan friends of Ferrán ; but it must be distinctly remembered that at that day there were practically no official statistics of this kind in the hands of any one. The official statistics collected under the orders of the Spanish Government were gotten together at a far later date.

"Upon the appointment of the Government at Madrid of the second official Spanish Commission to investigate the Ferrán question in the provinces where the inoculations were being practised, it was ordered that official statistics of inoculation should be collected in the usual manner ; that is to say, by the customary statistical officers of the Government. This second medical Commission was also accompanied by an independent statistical commission who were charged with the duty of forming statistics of those inoculations which were expected to be witnessed by the Medical Commission in their tour of investigation, and the report to the Spanish Government of this statistical commission is based exclusively upon the official statistics which they themselves collected.

"In estimating the value of the official character and the authority of the official statistics, which have *since the visit of the French Commission to Spain* been collected and published, the following circumstances should be taken into account: The provincial governments of Spain are somewhat peculiar, in that the civil governors change with the changes which take place in the Government at Madrid, so that the political constitution of the provincial governments is always a reflex of that of the central government at Madrid. Moreover, the political sentiment of the provincial government is also more or less perfectly reflected by the local governments of the towns of the province.

"The hostility of the Minister of the Interior at Madrid to Dr. Ferrán, and his attempts at the prevention of cholera by inoculation, is a well-known fact now generally admitted ; and the hostility which Dr. Ferrán met with from the civil governor of the province of Valencia was even greater than that manifested by the Minister of the Interior himself.

"The official statistics of the Ferrán inoculations are in the first place signed by the physicians of the locality; and in the next place by the judge of the municipal court, and sometimes also by the president judge of the judicial district, by the parochial priest, and by the mayor of the municipality, whose signatures and seals are attested by an authorized notary public.

"It must, therefore, be obvious that the charge made by the French Commission, which has been so constantly reiterated everywhere, that the public statistics of the anti-choleraic inoculations are void of official character and are to be regarded as *ex-parte* testimony of the partisans of Ferrán, cannot apply to official statistics which were collected under the supervision of the municipal authorities of the villages wherein the inoculations were performed, and attested not only by the local judicial officers and the parochial priests, but also by the political officers—that is to say, the secretaries and the mayors of the municipalities ; for it must be admitted that neither the political officers of the municipalities nor of the provincial governments, any more than the parochial priest, can reasonably be charged with being the partisans or friends of Ferrán—the Minister of the Interior continuing during the time of collection of these official statistics to be hostile to the claims of Ferrán. It therefore follows that the attack upon the statistics of the inoculations made by the French Commission, and so widely accepted by the medical world as conclusive, does not apply to the official statistics of which we are speaking. And, in view of this fact, the evidence as to the efficiency and harmlessness of the anti-choleraic inoculations should be re-examined. As I have already said, the results of the preventive inoculations of Ferran as set forth in the official statistics appear to very strongly support his claim of the protective value of the inoculations. In view of the great importance of this whole subject, I have determined to place these statistics in this report for the benefit of the readers of the English language, in order that they may judge for themselves of the facts as they appear to be recorded.

"From the Government statistics of cholera throughout the province of Valencia, it appears that among the villages invaded there were 62 attacks per one thousand of the population, and 31 deaths per thousand, which gives a mortality of 50 per cent of those attacked. It appears from analysis of the published official statistics of cholera in 22 towns where inoculation was performed the inhabitants were divided as follows: 104,561 not inoculated; 30,491 inoculated. Of the latter there were 387 attacks of cholera, or 12 per thousand, and 104 deaths, or 3 per thousand; the mortality of those attacked being 25 per cent. Of the former there were 8,406 attacks, or 77 per thousand, and 3,512 deaths, or 33 per thousand, being a mortality of those attacked of 43 per cent. It appears, therefore, that among the population of villages wherein anti-choleraic inoculations had been more or less extensively performed the liability of the inoculated to attacks of cholera was 6.06 times less than that of the non-inoculated, whilst the liability of the inoculated to death by cholera was 9.87 times less than that of the non-inoculated. These figures are based exclusively upon the data furnished by inoculations, the reinoculations being left out of consideration, because they are much less numerous, although from the records of the inoculations it would seem that the liability of attack, and especially of death by cholera, is many times less among them than among those inoculated a single time.

"The charge has also been made with respect to the published records of the inoculations that the hygienic and physical condition of the subjects of inoculation have not been sufficiently indicated in the records, and that the vast majority of those profiting by the opportunity to receive the anti-choleraic inoculations were of the middle and upper classes, and therefore not of that class of inhabitants who are notoriously most liable to attack and death from cholera. This criticism may have some justness as respects some, perhaps many, of the villages where inoculations were performed; but there are certainly many of the villages wherein the results of the inoculation seemed to be most positively in favor of the claim of Ferrán where this criticism cannot hold. I refer to villages wherein three-fourths or four-fifths of the inhabitants were inoculated, leaving only the fraction of the population non-inoculated. Even in the absence of any special notes indicating the social conditions and hygienic surroundings of the inoculated in these villages, it is ridiculous to assume that the vast majority of these were people of the middle and upper classes, and were therefore but little liable to attack and death by cholera. Any one acquainted with the character of the Spanish population as it exists in the rural villages will admit at once that the vast majority of this population consists of the wretched and the poor, who live under the most unhygienic and unsalubrious conditions, and therefore are of that class most liable to suffer from cholera.

"There is still another result of the preventive inoculations of Ferrán apparently shown by these statistics. I refer to the apparent marked shortening of the course of the epidemic after a large percentage of the inhabitants had become inoculated. It would seem, therefore, from analysis of the official statistics, that the practice of the anti-choleraic inoculation after the method of Ferrán, besides giving the subject inoculated a considerable immunity from attack and death by cholera, furnishes a means of bringing an epidemic rapidly to an end."

With reference to Haffkine's method of inoculation we cannot do better than to quote from a lecture which he gave in London, in 1893:

"In the research that I have done at the Pasteur Institute on vaccination against Asiatic cholera I have chosen for my starting-point the inoculation of the animal into the peritoneal cavity. Starting from this point I have worked out a method which permits the culture of the microbe in the animal organism in a state of purity during indefinite generations, the exaltation of it to a well-determined maximum of strength, and keeping it at the same degree of virulence for an unlimited period of time.

"This method is illustrated by three series of experiments which were the subject of our publications in the *Comptes rendus de la Société de Biologie* of Paris, and which are:

"1. Giving the first animal a dose larger than the fatal dose, and killing this animal in a sufficiently short space of time to be able to find the more resisting microbes.

"2. To expose the exudation taken from the peritoneal cavity to the air for several hours.

"3. Then to transfer this exudation to the next animal, of large or small size, according to the concentration of the exudation.

"In the hands of a number of other experimenters this method has given the same results and showed a perfect consistency.

"The properties of the virus which is obtained in this manner of cultivation are as follows: Upon intraperitoneal inoculation it kills guinea-pigs regularly in the space of about eight hours, and the fatal dose for this animal is reduced to about twenty times less than that which it would have been necessary to take for the microbe with which I started. The same inoculation kills rabbits and pigeons with a dose which would have been perfectly harmless at the beginning of the experiments. It kills guinea-pigs by intramuscular inoculation.

"The subcutaneous inoculation brings about the formation of a large œdema, which tends toward sequestration of a whole part of the cutaneous tissues and to the formation of a wide open wound, which is cured in from two to three weeks.

"The basis of anticholeraic vaccination is founded on the virus obtained in the manner we have just described.

"This virus, injected under the skin of a healthy animal, gives it, after several days, immunity from all choleraic contamination, in whatever manner this may arise; that is to say, if an animal that has been thus treated be taken, and an attempt made to infect it either by the digestive canal, by neutralization of the gastric juice and the injection of opium into the peritoneum, or by the introduction of the microbe into the intestines by the method of Nicati and Rietsch, or by intramuscular inoculation, or finally, by intraperitoneal injection, the most terrible of all, it resists, whilst the control animals succumb.

"Anticholeraic vaccination of animals in this manner is then definitely established. But the operation described cannot be, such as it is, applied to man. The wound following on the subcutaneous inoculation is terrible to look at, and, in all probability, extremely painful. Besides, although it does not in itself present any danger to the health of the individual, it exposes him to all the complications inseparable from an open wound.

"This power of producing necrosis of the cutaneous tissues has been removed from the exalted vaccine by cultivating it at a temperature of 39° C., and in an atmosphere constantly aërated. Under these conditions the first generations of the cholera microbe would die rapidly, in an interval of two to three days, and therefore care must be taken to sow them again in new media immediately before death, and after a series of generations of this kind a culture is obtained which, if injected under the skin of animals, even in exaggerated doses, produces only a passing œdema, and prepares the organism in such a manner that the injection of exalted virus, the definite vaccine, only produces a local reaction of the slightest description.

"VACCINATION BY FIXED VACCINE.

"The method of vaccination thus worked out comprises, then, two vaccines—a mild vaccine, obtained by weakening the fixed virus; and a strengthened vaccine, which is presented by the virus itself. It is easy to understand why, to obtain the weakened vaccine, we do not use an ordinary virus, but a virus the nature of which has been previously fixed in the laboratory. It is because the virus, such as is found in the natural state, especially when it has a saprophytic phase of development, presents such pathogenic differences that there is no certainty in its application. Respecting this we need only recall the story of variolization, and the great danger that an individual incurred when the infectious substance from a slightly attacked subject was transferred to him. The mildness or the gravity of an infection does not depend only on the veritable strength of the contagious substance, but upon the resistance of the individual from whom it is taken. Thus it happened that in taking vaccine lymph from a subject lightly affected, a very weak substance was sometimes produced, which was incapable of producing a protective action; and sometimes a lymph of such strength that it killed less resistant individuals. The great benefit of Jenner's discovery lay in that it precisely indicated a substance fixed by passages through animals, and of a virulence below that which is fatal to the human organism. Another example is given in the method of Toussaint of vaccination against anthrax, the first of its kind, which has been obliged to make way for the method of M. Pasteur, for the sole reason that the latter, based upon virus of a fixed nature, presented an absolute certainty in its results which was wanting in the other. Finally, in the history of cholera itself I may recall the attempt made in 1885 by Dr. Ferrán, of Barcelona, who, with the object of preserving the population of the Peninsula from the epidemic of cholera, made injections in his patients of the ordinary virus taken from dead bodies and cultivated in the laboratory. The statistics of the results obtained by this means showed such uncertainty that no one dared to recommend this operation to his country in spite of the very numerous trials made in Spain.

"The possibility of treating the animal organism by vaccines of an absolutely fixed nature, prepared by means of special operations, constitutes, on the contrary, the basis of the Pasteurian method, and here lies the whole secret and the sole guarantee of the success of its application.

"APPLICATION OF THE METHOD TO MAN.

"The method of anticholeraic vaccination, worked out by experiments on guinea-pigs, was tried upon rabbits and pigeons before it was applied to man. These animals were chosen in order to have subjects very differently organized, and in order to be able to generalize the conclusions, and to be able to extend them to the human organism.

"The result obtained on all these animals being absolutely the same, it was decided to apply the operation to man.

"The symptoms produced by this operation have been described in several scientific magazines. The method has been tried at Paris, Cherbourg, and at Moscow, on about fifty persons of both sexes, between the ages of nineteen and sixty-eight, of French, Swiss, Russian, English, and American nationality.

"In every case the method has shown itself absolutely harmless to health, and the symptoms that it evoked were a rise in temperature, a local sensitiveness at the place of inoculation, and the formation of a transitory œdema at the same place. The first sensations are felt about two or three hours after inoculation; fever and general indisposition disappear after twenty-four to thirty-six hours; the sensitiveness and œdema last, gradually dying away in from three to four days. The symptoms following the second inoculation were generally rather more marked, but of shorter duration. The whole recalls the sensation of a bad cold in the head, lasting about one or two days.

"The microbes introduced under the skin do not propagate, but after a certain time they die and disappear. It is the substances which they contain, and which are set free when they die, that act upon the animal organism and confer immunity upon it. It is found that the same result can be obtained if the microbe be killed before inoculation, and if their dead bodies only be injected. Thus I have been enabled to prepare vaccines preserved in weak solutions of carbolic acid. In this the microbes die at the end of several hours, and the vaccine so prepared has been found still efficacious six months after its preparation. It is evident that there is much advantage in this state of preservation of the microbes. They can be used by persons having no bacteriological training, and the absence of every living organism makes them perfectly safe. The carbolic acid that they contain preserves them against any invasion of other microbes. Finally, as they can be kept for several months, their preparation can be entrusted to a central laboratory, whence the vaccine *ampoules* can be sent out to operators. But it may be presumed that immunity given by these preserved vaccines will not equal in persistency that produced by living ones, and as the method is not yet backed up by established statistics, it is better that vaccinations should be done as much as possible with living virus, so as to obtain the most conclusive results.

"As to the length of time that immunity produced by living vaccine lasts, we have not yet at the laboratory animals that have been inoculated at a very distant date; those upon which we experimented dated from, at most, four months and a half. At the end of this time their immunity was found to be still perfect, and we do not despair of its lasting much longer yet.

"HARMLESSNESS OF THE METHOD.

"The inoculations upon man, added to the hundreds of experiments that we have made upon animals, testify to the perfect harmlessness of these operations, and there is no difficulty in proving their efficacy by experiment, no matter on what species of animal. We have taken twelve guinea-pigs, and vaccinated six of them with vaccines preserved in carbolic acid since September 8th last. Yesterday, at five o'clock, six days after the first vaccination, we injected into the peritoneal cavity of all the non-vaccinated animals a fatal dose of virus, and into the vaccinated animals we injected a double dose. The six vaccinated animals are perfectly well, while of the others two have already died of choleraic poisoning, two are very ill, and the others will certainly soon become so. But it is evident that I cannot perform a like experiment on man (but, however, this would be the only means of being able to give a definite experimental demonstration)."

Further details as to the method are given by Woodhead in the "Edinburgh Hospital Reports," as follows:

"In order to be absolutely certain that the virus is pure, M. Haffkine makes cultivations before each inoculation of the human subject, by Roux and Yersin's method, one devised for the separation of the diphtheria bacillus. A small drop of the *virus exalté* is taken on a spatula-shaped needle, and streak after streak is made with the flat of this needle on the surface of the agar in the tubes, a couple of tubes being used, so that twelve streaks perhaps, in all, are made without the needle being recharged; in the earlier streaks, of course, the seed bacilli are so close together that a continuous line of colonies makes its appearance; but along the course of the later streaks, colonies, with distinct intervals between them, are developed; part of one of these is examined under the microscope, in order to determine that it is made up only of comma bacilli, and then the other part is used for seed material for a tube culture preparatory to inoculation.

"The inoculation itself is an exceedingly simple process; the needle and the syringe are boiled; the tube containing the material to be used for inoculation receives a syringeful or pipetteful of sterilized beef broth, then with a platinum needle the culture is thoroughly mixed with this broth, so that a kind of emulsion is prepared; this emulsion is drawn up in a sterilized pipette, and is then passed into a sterilized conical glass covered with sterilized paper. If a sixth of the culture is to be introduced, two more syringefuls or pipettefuls of broth are to be added, so that we now have three in all; if an eighth, three are added, and so on; the whole is mixed, and then half a syringeful is taken for use for each patient. In inoculating, the skin, just above the crest of the ilium, is thoroughly cleansed with five-per-cent solution of carbolic acid, the attenuated virus is inoculated on the left side, and then after an interval of four or five days the second vaccine, or the more virulent form, is inoculated on the right side. After inoculation everything that has been used is thoroughly boiled, the skin of the patient is again washed with five-per-cent carbolic acid, and the table is washed down with the same solution."

Haffkine commenced his experiments on man by inoculating himself, and has repeated the inoculation three times. He next inoculated about fifty individuals in Paris, Cherbourg, and Moscow, and demonstrated in a satisfactory way that the inoculations are without danger.

A first inoculation in an unprotected person is said to give rise to some malaise and febrile reaction, to pain and tumefaction at the point of inoculation, and swelling of the neighboring glands. The second inoculation with a strong virus, made after an interval of six days, causes also some elevation of temperature, but no swelling at the point of inoculation. This slight reaction from a strong virus is supposed to be satisfactory evidence of a certain degree of immunity as a result of the first inoculation.

The results of the protective inoculations by Haffkine's method, which have been practised in India, indicate that these inoculations have a real value, but that immunity is not immediately established, and consequently that during an epidemic a certain number of fatal cases may be expected among the inoculated as well as among the non-inoculated. This is illustrated by the results of inoculations made among the prisoners in Gaya jail (1894), reported by Surgeon-Major Macrae, I.M.S., from whose report we quote as follows:

"Cholera broke out in Gaya jail on the 9th of July, and from that date until 2d August 34 cases occurred, with 20 deaths, there being on date of first attack 422 prisoners in jail. The disease was clearly traceable to importation, but its diffusion among the prisoners was a question of much greater difficulty. The sanitary condition of the jail is excellent; it was built quite recently, on the latest plans, and is generally considered a model jail. The water supply, which is from a well, is of excellent quality and protected from pollution, and it is believed that the spread of the disease was largely due to the agency of flies finding access to food and milk after being in contact with cholera poison, and contaminating them. From the 9th to the 17th July six cases occurred, with five deaths.

"Many of the prisoners on being told about preventive inoculation wished to be inoculated, and M. Haffkine, who had previously been communicated with, and whose zeal and enthusiasm in the cause that he so well advocates are beyond praise, arrived here on the 18th July, and in the presence of Surgeon-Colonel Harvey, who kindly assisted, and myself, inoculated 147 prisoners, and on the 19th 68, making a total of 215 out of 433 present in the jail on that date.

"Being purely voluntary, no selection of prisoners was possible; but all classes in the jail were represented, male and female, old and young, habituals and less frequent offenders, strong and weakly, convalescent and even hospital patients sent their representatives. No difference of any kind was made between inoculated and non-inoculated prisoners; they were under absolutely identical conditions as regards food, water, accommodation, etc., in short, in every possible respect.

"As, owing to the progress of the epidemic, a large number of prisoners were removed from the jail into camp, it will be found convenient to consider the effect produced by the anticholera inoculation under three headings:

"(*a*) *The first* will include the period from the 18th July, the date of first inoculations, to the 24th July, the date on which final reinoculations were made, and refers to all the prisoners.

"(*b*) *The second* concerns the prisoners who remained in jail after the majority were removed into camp, and comprises the period from 25th July to 2d August, on which date the final case occurred among this body of prisoners.

"(*c*) *The third* refers to the body of prisoners who were moved into camp on 25th July, and includes the period between that date and 1st August, when the final case occurred among this body.

	Average present.	Cholera.	Percentage of average strength.	Deaths.	Percentage of average strength.	Percentage of deaths to cases.
			No. I.			
Inoculated.............	211.2	5	2.37	4	1.89	80.0
Not inoculated.........	209.0	7	3.34	5	2.39	71.42
			No. II.			
Inoculated.............	32.5	1	3.07	Nil.	Nil.	Nil.
Not inoculated.........	48.55	7	14.42	3	6.18	42.86
			No. III.			
Inoculated.............	171.42	2	1.16	1	0.58	50.0
Not inoculated.........	146.5	6	4.09	2	1.36	33.33

"The conclusions to be drawn from the results above recorded appear to me to be that for the first few days the inoculations have scarcely any protective influence: then their effect seems to gradually increase. M. Haffkine in his publications has laid stress on the fact that he anticipates a period of ten days would elapse from date of first inoculations before the full effect would be obtained.

	DURING THE FIRST FIVE DAYS AFTER FIRST INOCULATION.		FIRST THREE DAYS AFTER SECOND INOCULATION.		LAST SIX DAYS.	
	Cases.	Deaths.	Cases.	Deaths.	Cases.	Deaths.
Inoculated......	5	4	3	1	Nil.	Nil.
Not inoculated...	7	5	5	3	8	2

"Further observations are necessary to prove whether the inoculations as now practised will prove of lasting benefit; the results obtained in Gaya jail seem to me to justify the conclusion that their temporary beneficial effect is undoubted.

"I have been informed by M. Haffkine that he proposes to introduce a certain modification of his method, with the object of affording protection to patients during the ten days necessary for the action of his vaccines. I think there is every reason to believe that better results would have been obtained here had the inoculations been performed at an earlier period instead of during the epidemic."

In a paper published in the *British Medical Journal* (January 26th, 1895), Haffkine gives the following summary of his inoculations in India:

"TABLE SHOWING THE TOTAL NUMBER OF PERSONS ON WHOM OBSERVATIONS HAVE BEEN MADE IN CALCUTTA, GAYA, CAWNPORE, AND LUCKNOW.

	Number.	Cases.	Percentage of Cases to Strength.	Deaths.	Percentage of Deaths to Strength.
Non-inoculated	1,735	174	10.63	113	6.51
Inoculated..................	500	21	4.20	19	3.80
Total..................	2,235	195		132	

Other methods of producing immunity in man have been proposed, and experiments indicate that this may be accomplished through the digestive tract by the ingestion of considerable quantities of sterilized cultures. Thus Klemperer (1892) has obtained results which seem to show that immunity in man may be induced, not only by the subcutaneous injection of virulent cultures, but also by the subcutaneous injection of the milk of immunized goats and by the ingestion of cultures sterilized by heat. The degree of immunity, as determined by the activity of the blood serum of the immune individual for the protection of guinea-pigs, is considerably less, however, than when repeated injections of virulent cultures have been made. The blood serum of individuals made immune by the last-mentioned method is said by Klemperer to protect guinea-pigs when injected into the cavity of the abdomen in the dose of 0.005 cubic centimetre. And the injection of five cubic centimetres of milk from an immunized goat is said to confer such an immunity that 0.25 cubic centimetre of blood serum from the immune individual is sufficient to protect a guinea-pig from cholera cultures.

Sawtschenko and Sabolotny (1893), as a result of a series of experiments made upon themselves and laboratory assistants, arrive at the following conclusions:

"1. After the ingestion of sterilized (by heat) and subsequently carbolized agar cultures of cholera bacteria the serum of man acquires an immunizing property as regards the cholera vibrio.

"2. As a result of the ingestion of sterilized agar cultures the individual is protected from infection with virulent cultures of the cholera vibrio by way of the intestine.

"3. The discharges of individuals immune against cholera, and to all outward appearance in perfect health, may contain a great number of cholera vibrios (in case they are in any way introduced into the intestine) and may thus serve to propagate the malady."

DIPHTHERIA.

According to Roux and Yersin, "attenuated varieties" of the diphtheria bacillus may be obtained by cultivating it at a temperature of 39.5° to 40° C. in a current of air; and these authors suggest that a similar attenuation of pathogenic power may occur in the fauces of convalescents from the disease, and that possibly the similar non-pathogenic bacilli which have been described by various investigators have originated in this way from the true diphtheria bacillus. These authors further state, in favor of this view, that from diphtheritic false membrane, preserved by them in a desiccated condition for five months, they obtained numerous colonies of the bacillus in question, but that the cultures were destitute of pathogenic virulence. They say:

> "It is then possible, by commencing with a virulent bacillus of diphtheria, to obtain artificially a bacillus without virulence, quite similar to the attenuated bacilli which may be obtained from a benign diphtheritic angina, or even from the mouth of certain persons in good health. This microbe, obtained artificially, resembles completely the pseudo-diphtheritic bacillus; like it, it grows more abundantly at a low temperature; it renders bouillon more rapidly alkaline; it grows with difficulty in the absence of oxygen."

Subcutaneous inoculations in guinea-pigs of a small quantity of a pure culture of the bacillus (0.1 to 0.5 cubic centimetre of a bouillon culture) cause death in from one to four or five days. The usual changes observed at the autopsy are—

> "An extensive local œdema, with more or less hyperæmia and ecchymosis at the site of inoculation, frequently swollen and reddened lymphatic glands, increased serous fluid in the peritoneum, pleura, and pericardium, enlarged and hemorrhagic suprarenal capsules, occasionally slightly swollen spleen, sometimes fatty degenerations in the liver, kidney, and myocardium. We have always found the Löffler bacilli at the seat of inoculation most abundant in a grayish-white, fibrino-purulent exudate present at the point of inoculation, and becoming fewer at a distance from this, so that the more remote parts of the œdematous fluid do not contain any bacilli" (Welch and Abbott).

The authors quoted agree with Löffler and others in stating that the bacillus is found only at the point of inoculation. In all cases their cultures from the blood and from the various organs gave a negative result.

Rabbits are not so susceptible, and may recover after the subcutaneous inoculation of very small doses, but usually die in from four to twenty days when two to four cubic centimetres of a bouillon culture have been introduced beneath the skin. In these animals, also, there is an extensive local œdema, an enlargement of the neighboring lymphatic glands, and a fatty degeneration of the liver. Roux and Yersin have shown that in rabbits, when death does not ensue too quickly, paralysis of the posterior extremities frequently occurs, thus completing the experimental proof of the specific pathogenic power of pure cultures of this bacillus.

Similar symptoms are produced in pigeons by the subcutaneous inoculation of 0.5 cubic centimetre or more, but they commonly recover when the quantity is reduced to 0.2 cubic centimetre (Roux and Yersin).

The rat and the mouse have a remarkable immunity from the effects of this poison. Thus, according to Roux and Yersin, a dose of two cubic centimetres, which would kill in sixty hours a rabbit weighing three kilogrammes, is without effect upon a mouse which weighs only ten grammes.

Old cultures are somewhat less virulent than fresh ones, but when replanted in a fresh culture medium they manifest their original virulence. Thus a culture upon blood serum which was five months old was found by Roux and Yersin to kill a guinea-pig in five days, but when replanted it killed a second animal of the same species in twenty-four hours.

Evidently a microörganism which destroys the life of a susceptible animal when injected beneath its skin in small quantity, and which nevertheless is only found in the vicinity of the point of inoculation, must owe its pathogenic power to the formation of some potent toxic substance, which, being absorbed, gives rise to toxæmia and death. This inference in the case of the diphtheria bacillus is fully sustained by the results of experimental investigations. Roux and Yersin (1888) first demonstrated the pathogenic power of cultures which had been filtered through porous porcelain. Old cultures were found by these experimenters to contain more of the toxic substance than recent ones, and to cause the death of a guinea-pig in a dose of two cubic centimetres in less than twenty-four hours. The filtered cultures produced in these animals the same effects as those containing the bacilli—local œdema, hemorrhagic congestion of the organs, effusion into the pleural cavity. Somewhat larger doses were fatal to rabbits, and a few drops injected subcutaneously sufficed to kill a small bird within a few hours. In their second paper (1889) the authors mentioned state that so long as the reaction of a culture in bouillon is acid its toxic power is comparatively slight, but that in old cultures the reaction is alkaline, and in these the toxic potency is greatly augmented. With such a culture, filtered after having been kept for thirty days, a dose of one-eighth of a cubic centimetre injected subcutaneously, sufficed to kill a guinea-pig; and in larger amounts it proved to be fatal to dogs when injected directly into the circulation through a vein.

The same authors, in discussing the nature of the poison in their filtered cultures, infer that it is related to the diastases, and state that its toxic potency is very much reduced by exposure to a comparatively low temperature—58° C., for two hours—and completely destroyed by the boiling temperature—100° C., for twenty minutes. It was found to be insoluble in alcohol, and the precipitate obtained by adding alcohol to an old culture proved to contain the toxic substance. Löffler also has obtained, by adding five volumes of alcohol to one of a pure culture, a white precipitate, soluble in water, which killed rabbits in the dose of 0.1 to 0.2 gramme when injected beneath the skin of these animals. It gave rise to a local œdema and necrosis of the skin in the vicinity of the point of inoculation, and to hyperæmia of the internal organs. This deadly *toxin* appears to be an albuminoid substance, but its exact chemical composition has not yet been determined.

Brieger and Fränkel (1891) obtained results corresponding with those previously reported by Roux and Yersin. Their researches showed that the toxic substance contained in diphtheria cultures is destroyed by a temperature of 60° C.; that it is soluble in water, and insoluble in alcohol; that it does not pass through a dialyzing membrane, and has not the chemical characters of the ptomaïnes or toxins, but is an albuminous body—a toxalbumin. It was obtained by the authors named by precipitation with slightly acidified (acetic acid) alcohol; the precipitate, after being washed in a dialyzer and dried in a vacuum at a temperature of 40° C., was a snow-white, amorphous, crumbling mass.

Wassermann and Proskauer (1892) found that the alcoholic precipitate from diphtheria cultures contains two different substances, which are distinguished by their different degrees of solubility in dilute and absolute alcohol; both, however, give the usual reactions of albuminous bodies, and pass very slowly through a dialyzing membrane. Only one of these substances possesses toxic properties.

After the removal of peptone and globulin from the filtered cultures, these were evaporated and a precipitate obtained of one of the albuminous substances by means of sixty to seventy per cent alcohol. The other substance remained in solution, and was subsequently obtained by precipitation with absolute alcohol. The substance first obtained by this method is toxic, and the other precipitate is not. The authors named succeeded in killing rabbits with the toxalbumin obtained in this way, but were not able to produce immunity in these animals by the injection of non-fatal doses. Fränkel (1891) had previously reported his failure to immunize guinea-pigs by the injection of the dry precipitate, obtained in his experiments from diphtheria cultures; but when filtered cultures, or cultures sterilized by heat (55° C. for one hour), were injected into these animals, they showed an increased resistance to the pathogenic action of virulent cultures. Still better results were obtained when ten cubic centimetres of a bouillon culture, heated to 100° C., were injected subcutaneously, but still this method was not entirely reliable. But true immunity was established by injecting into the peritoneal cavity ten to twenty cubic centimetres of a bouillon culture heated to from 65° to 70° C. for one hour. The immunity was not fully established until about fourteen days after the protective inoculation. Fränkel arrives at the conclusion that the cultures must contain an immunizing substance as well as a toxic proteid, as the diphtheria toxalbumin is destroyed by the temperature (65° to 70° C.) used in the preparation of his cultures for producing immunity.

Behring, in the same year (1891), commenced his experiments upon diphtheria immunity. Guinea-pigs were made immune by the use of sterilized cultures, and by inoculations with virulent cultures, four weeks old, to which iodine terchloride had been added in the proportion of 1:500—the mixture was allowed to stand for sixteen hours. Animals were also immunized by injecting beneath the skin a virulent culture of the bacillus, and then treating them with subcutaneous injections of iodine terchloride (two cubic centimetres), which was thrown under the skin for three days in succession in the vicinity of the point of inoculation. The guinea-pigs treated in this way remained sick for some time, but finally recovered and were subsequently immune. Still better results were obtained when rabbits were subjected to the same treatment. The animals were immune against the toxic action of sterilized cultures, as well as against infection by virulent diphtheria bacilli.

In subsequent experiments (1892) Behring and Wernicke used cultures which had been attenuated by contact with iodine terchloride for from thirty-six to forty-eight hours, and proved that the method could be successfully employed in immunizing sheep; and the fact was ascertained that blood serum from an immune animal could be used with success in arresting diphtheritic infection in susceptible animals. To preserve the serum, which they obtained from immunized sheep, rabbits, and guinea-pigs, they added to it 0.5 per cent of pure carbolic acid. For producing immunity they found that a smaller amount of serum was required than was necessary for the cure of an animal already infected. If the injection was made immediately after infection, from one and a half to two times the amount was required; eight hours after infection the amount was three times as great, and twenty-four to thirty-six hours after infection the dose required was eight times the immunizing dose.

The immunizing value of blood serum from different animals was estimated by finding the smallest dose which would protect an animal from fatal infection by the minimum lethal dose of a culture the toxic potency of which had been carefully determined. The value is expressed in figures which give the proportion required compared with the body weight of the animal. Thus an immunizing value of one hundred would mean that one gramme of the serum is sufficient to protect an animal weighing one hundred grammes from the fatal effect which would be produced in a control animal of the same weight by infection with a virulent culture of the diphtheria bacillus in the minimum doses required to produce this result. The cultures employed are made in bouillon containing one per cent of peptone; they are inoculated from agar cultures and are kept in the incubating oven for two days. Cultures prepared in this way were found to be quite uniform in their pathogenic virulence as tested upon guinea-pigs. But when cultures are kept for some time there is an increase in virulence. Thus a culture obtained from a fatal case of diphtheria which in 1890 killed guinea-pigs in three to four days, when injected subcutaneously in the dose of 0.1 cubic centimetre (two-days-old bouillon culture), at the end of a year was fatal to these animals in the dose of 0.025 cubic centimetre. This increase in virulence is ascribed to the fact that the cultures were renewed at long intervals.

More recently (1894) Behring has fixed a standard for what he calls a normal therapeutic serum. This is a serum which when injected into guinea-pigs in the proportion of 1:5,000 of body weight saves the animal from the fatal effects of ten times the minimum dose of a culture in bouillon, two days old, which would kill a control animal not treated.

In a subsequent communication (November, 1894) Behring states his conclusion that for producing immunity in man, one hundred and fifty normal antitoxin units should be given, instead of sixty as he had previously recommended.

The serum manufactured under his direction is said (September, 1894) to be of two kinds—one, obtained from the horse, has a value of sixty normal antitoxin units; the other has a value of one hundred and forty units. Of the weaker serum Behring says experience has demonstrated that for children under ten years of age ten cubic centimetres is sufficient to arrest the progress of the disease and effect a cure if given within two or three days from the outset of the attack. For producing immunity in children subject to infection, one-tenth of this amount (one cubic centimetre) is said to be sufficient. Of the stronger serum one cubic centimetre is sufficient to arrest the disease during the incubation period; and, according to Behring, out of one hundred cases treated during the first forty-eight hours with a single therapeutic dose (ten cubic centimetres of serum having a value of sixty, equals six hundred normal units), not five will die. The later the treatment is commenced the larger will be the dose required. Behring further states that the diphtheria antitoxin has no injurious effect upon animals in the largest doses that have been employed, and that aside from its antitoxic power its properties are entirely negative so far as living animals are concerned.

Aronson (1893), in experiments on dogs, succeeded in producing immunity by the use of attenuated cultures, or of cultures to which formaldehyde had been added; also by feeding the animal large quantities of diphtheria bouillon; and, finally, by injection of the blood of naturally immune animals (white rats) into which large quantities (ten cubic centimetres) of a virulent culture had been injected. Two months after receiving several such injections it was found that 0.2 gramme of blood-serum from the rat sufficed to save a guinea-pig from fatal infection. In experiments on dogs an immunity was established in six weeks by the injection of a large amount of a virulent culture. Its serum had a value of 1:30,000, *i.e.*, 0.01 cubic centimetre of this serum sufficed to protect a guinea-pig weighing three hundred grammes. From one hundred grammes of this serum Aronson claims to have obtained 0.8 gramme of a substance which had a value of 1:500,000, as tested in the treatment of an animal which had received ten times the minimum fatal dose of a two-days' bouillon culture. A ten-per-cent solution of this substance had, therefore, ten times the value of Behring's "normal serum." The precipitated antitoxin was soluble in water, and more readily so in a slightly alkaline solution, and gave all the reactions of an albuminous body. When dried in vacuo at

40° C., and then heated to 102° C., it still retained its antitoxic potency.

Ehrlich, Kossel, and Wassermann (1894) have made experiments upon goats, which they found very susceptible to the action of the diphtheria poison. Sterilized cultures were first injected in gradually increasing amounts, and later virulent cultures. In this way they obtained a serum which has a value sixty times that of Behring's "normal serum." In a subsequent communication (1894) Wassermann gives an account of his experiments with the milk of immunized goats, which contains the antitoxin in considerable quantity, and from which it was obtained in a concentrated form by the following method: The milk is obtained in sterilized vessels and twenty cubic centimetres of normal hydrochloric acid are added to each litre; a sufficient quantity of rennet is then added to coagulate the casein, and this is separated from the liquid, which is then shaken up with chloroform for some time. The liquid is now allowed to stand in order that the butter, which has been dissolved by the chloroform, may sink to the bottom. The clear liquid is then decanted and the antitoxin precipitated from it by means of ammonium sulphate (thirty to thirty-three per cent). The precipitate is rapidly dried upon porous porcelain plates, in vacuo, and then dissolved in water in the proportion of ten parts for one hundred of milk first employed—a concentration to one-tenth. Of this solution 0.125 cubic centimetre was found to neutralize 0.9 cubic centimetre of a toxin which killed guinea-pigs weighing five hundred grammes in the dose of 0.1 cubic centimetre. This toxin was an old bouillon culture of the diphtheria bacillus to which 0.5 per cent of carbolic acid had been added to preserve it. In a communication of the same date Ehrlich and Wassermann report that they have for some time had a cow immunized to such a degree that one cubic centimetre of its milk protects guinea-pigs from the fatal effects of 0.9 cubic centimetre of the above-mentioned toxin. The antitoxic value of the milk of an immunized cow or goat, as compared with that of its blood, is estimated by Ehrlich and Wassermann as from 1:15 to 1:30—usually about 1:20.

Aronson, in testing his antitoxin, uses a bouillon culture of the diphtheria bacillus two and one-half months old, which he preserves by the addition of 0.3 per cent of trikresol. He finds that the immunity which results from injections of the antitoxin is established at once; that it is not accompanied by any reaction or symptom of sickness; and that it is of comparatively short duration.

As a result of extended experiments made at the Pasteur Institute in Paris, Roux has perfected the following method for the production of an antitoxin suitable for use in the treatment of diphtheria in man. The horse has been found the most suitable animal for this purpose, on account of his slight susceptibility and the ease with which a high degree of immunity can be established; and because of the large amount of blood that may be drawn without injury to the animal. Roux prepares his toxin by cultivating the diphtheria bacillus in a slightly alkaline bouillon made from beef and containing two per cent of peptone and 0.5 per cent of sodium chloride. This medium is placed in flat-bottomed flasks, and should not be more than half an inch in depth. Two glass tubes pass into the flask, which serve for inlet and outlet tubes to be used in passing a current of air over the cultures. This is commenced when the growth is fairly started, at the end of twenty-four hours, and the air should be moist to prevent the evaporation of the culture. In Roux's laboratory a flask is used which has a tube attached to one side, about an inch from the bottom, and which is known as a Fernback flask. A flocculent deposit falls to the bottom and gradually accumulates for about a month. This consists of bacilli which have for the most part lost their vitality and are undergoing degeneration. At the end of thirty days, during which time they are kept in an incubating oven at a temperature of 37° C., the cultures are passed through a Pasteur-Chamberland filter, and 0.5 per cent of carbolic acid may be added in order to preserve them. This filtrate is so toxic that a dose of 0.1 cubic centimetre will kill a guinea-pig weighing five hundred grammes in less than forty-eight hours. A healthy horse is selected and receives at first a dose of 0.5 cubic centimetre of the filtered culture (or of the clear fluid obtained from a culture by decantation, and containing 0.5 per cent of carbolic acid). The dose is gradually increased at intervals of a few days, and is followed each time by some febrile reaction and tumefaction at the point of inoculation. When the reaction is excessive, a little Gram's solution is added to the following dose. The usual plan of treatment is stated by Kinyoun as follows:

> "First day, 1 to 2 c.c. of pure toxins, of which 1 to 10 c.c. fatal to a 500-gm. guinea-pig; eighth day, 1 c.c.; fourteenth day, 1.5 c.c.; twentieth day, 2 c.c.; twenty-eighth day, 3 c.c.; thirty-third day, 5 c.c.; thirty-eighth day, 8 c.c.; forty-third day, 10 c.c.; forty-seventh day, 20 c.c.; fifty-first day, 30 c.c.; fifty-sixth day, 50 c.c.; sixty-second day, 50 c.c.; sixty-eighth day, 60 c.c.; seventy-fourth day, 100 c.c.; eightieth day, 250 c.c.; eighty-eighth day, 250 c.c.
>
> "When the first injections are given there is quite a marked local and general reaction to the poison; there is an œdema at the point of the injection, which is followed by a distinct inflammatory process—hard in the centre and soft and œdematous at its periphery. The general reaction is manifested by a rise in the temperature, 1° to 2° C., loss of appetite, and occasionally cramps. The reaction must be taken as the guide in the future dosage, and a sufficient

time must be allowed to elapse between the injections for the complete recovery from the general and local effects. As the quantity of the toxins is increased the general effects generally decrease, perhaps a rise of a degree for twenty-four hours. The local effect partakes more of an œdema, and has the character of an inflammation.

"At a certain stage, usually after two months' treatment, when fifty to sixty cubic centimetres can be injected without harm, there is no general reaction, but a large œdema at the site of the injection, which disappears within from twenty-four to forty-eight hours. Toward the last, even when two hundred to three hundred cubic centimetres are given, there is only an enormous œdema, which disappears within from twelve to eighteen hours. When these inordinately large quantities can be given with only a local reaction being manifest, the horse has come well under the influence, and the blood will be found to be rich in the antitoxin.

"There is a curious fact well worth noting: At the end of the second month of treatment, when the horse can bear as much as fifty to sixty cubic centimetres of the toxins without discomfort, the blood will be found to contain but little of the antitoxin. The antitoxin only appears after repeated stimulation of the cells (?) by the large and frequent doses of the toxins."

The subcutaneous injections do not yield a serum as rich in the antitoxins as when the toxins are injected directly into the blood current. When it is desired to do this, toward the last of the treatment the toxins are injected directly into the jugular vein. The process is tedious and requires a longer time, and for practical purposes has not been found so satisfactory as the simple subcutaneous injection. The strength of the serum is tested by using young guinea-pigs of five hundred grammes weight. One gramme of the serum usually will protect fifty thousand grammes of guinea-pig against a fresh virulent culture of the *Bacillus diphtheriæ*. This is the strength that is used in the hospitals. By the intravenous injections a serum of the protective strength of 1:100,000 can be obtained.

When fully immunized from six to eight litres of blood may be taken from a horse at one time, but as a rule it is better not to take more than three. The blood is drawn from the jugular vein, by means of a small trocar and cannula, into wide-mouthed bottles having a capacity of 2.5 litres; these are placed in an ice chest for twenty-four hours to give time for the separation of the serum, which is then transferred to smaller receptacles for preservation.

The dose of serum prepared in this way, when used to protect from diphtheria infection, is five cubic centimetres for a child under ten years of age, and ten cubic centimetres for older children. This does not afford an absolute protection, but is believed to be generally effective, and in case of failure the attack is said to be of a mild character. The curative dose of Roux's serum is twenty cubic centimetres for children, and thirty to forty cubic centimetres for patients over fifteen years of age. The larger dose is divided and given, at the same time, by subcutaneous injection in two places. Antiseptic precautions are taken in giving these injections, and a little absorbent cotton is placed over the puncture.

FOOT-AND-MOUTH DISEASE.

This is an infectious disease of cattle, sheep, goats, and swine, the etiology of which, so far as the specific infectious agent is concerned, has not been determined.

The extent to which the disease in question prevails in some parts of Europe is shown by the statistics for 1891 of the prevalence of this disease in Germany. According to the Reichsseuchenbericht it prevailed most extensively in the southern portion of Germany. The total number of infected farms was 47,865; the total number of infected cattle was 394,640; of sheep, 240,904; of goats, 3,378; of swine, 182,208. Behla (1892) has made inoculation experiments with the filtered saliva of infected cattle to which he added one to two per cent of carbolic acid, and claims to have produced immunity in young pigs and lambs. The duration is not, however, very long even in animals which have recovered from an attack of the disease—said to be from six months to three years—and a practical method of restricting the disease by means of protective inoculations has not as yet been introduced.

GLANDERS.

The toxic substances produced in cultures of the glanders bacillus when concentrated in the form of a glycerin extract constitute the so-called malleïn, which has been extensively used in the diagnosis of glanders in horses. As is the case when animals infected with tuberculosis are inoculated with tuberculin, animals infected with glanders have a decided rise of temperature after receiving a sufficient dose of malleïn beneath the skin.

Babes (1892) reports that the toxic substance in cultures of the glanders bacillus may be obtained by precipitation with alcohol; and that malleïn obtained from filtered cultures to which glycerin has been added, or the alcoholic precipitate, may be successfully used for protecting susceptible animals against glanders infection or for curing the disease after infection. He has demonstrated the therapeutic value upon guinea-pigs and upon two horses which are said to have been cured of chronic glanders. When large and repeated doses are injected into healthy animals they produce nephritis and general marasmus. The action upon horses infected with glanders is very marked and small doses may even cause death.

Kresling (1892) recommends potato cultures as preferable to bouillon cultures for the preparation of malleïn. The potatoes are to be washed, before sterilization, in a five-per-cent bicarbonate of soda solution, "until the wash-water remains clear." They are then cooked for an hour and twenty minutes. After planting upon the surface glanders bacilli from a previous culture they are placed in an incubator at 36° to 36.5° C., with provision to prevent them from becoming dry. At the end of two weeks the growth is removed with a platinum spatula and added to nine parts of water, in which it is well mixed by rubbing. It is then allowed to stand for twenty-four hours, after which it is sterilized for fifteen minutes at 110° C. (a lower temperature would no doubt answer quite as well). After cooling it is passed through a Chamberlain filter by means of a pressure of six atmospheres. The filtrate is then carefully evaporated over a water-bath to one-fourth its volume, and to this concentrated extract glycerin is added in the proportion of one part to two. The mixture is again sterilized in the autoclave at 110° C. When injected into healthy horses in the dose of two cubic centimetres this malleïn does not cause an elevation of temperature exceeding 0.5° to 0.8°. But one cubic centimetre injected into a horse having glanders causes its temperature to mount to 40° C., and at the point of inoculation a considerable swelling is developed which lasts from four to six days—in healthy horses a swelling the size of a man's fist is developed at the point of inoculation, which disappears within twenty-four hours.

In Pasteur's laboratory, according to Nocard (1892), malleïn is prepared as follows: The glanders bacillus is first made so virulent by successive inoculations in susceptible animals that it will kill a rabbit or a white mouse in a few hours. This virulent bacillus is cultivated in glycerin-peptone-flesh-infusion (five per cent of glycerin and five per cent of peptone). The cultures are kept in the incubating oven for four weeks at a temperature of 31° C., and then sterilized in the autoclave at 110° C. They are then filtered through paper and evaporated, in vacuo, over sulphuric acid, at a low temperature, to one-tenth of the original volume. The result is a syrup-like, dark-brown, strong-smelling liquid, which is about one-half glycerin. This can be preserved in a cool and dark place for a long time. When it is to be used nine parts of a 0.5-per-cent solution of carbolic acid are added to one part of the glycerin extract. The concentrated extract, when injected into a healthy horse in the dose of one-half to one cubic centimetre, causes a local swelling which disappears after two or three days. The temperature of the body is elevated from 1.5° to 2° C. as a result of the injection, and there are chilliness, loss of appetite, and debility. When the diluted malleïn is injected in healthy animals in the dose of 2.5 cubic centimetres no reaction occurs. On the other hand, this dose causes an intense febrile reaction in horses with glanders. There is a chill followed by an elevation of temperature amounting to 2° to 3° C., accompanied by dyspnœa and great debility; in some cases the animal dies as a result of the inoculation.

For the preparation of the active substance in a dry condition, Foth gives the following directions: The cultures are evaporated at a temperature not exceeding 80° C. to one-tenth of their volume, and filtered. The clear and thick, dark-brown liquid is then slowly dropped into absolute alcohol (twenty-five to thirty parts) with constant stirring. A flaky, white precipitate is thrown down, and accumulates as a pale yellow mass upon the sides and bottom of the vessel. After standing for twenty-four hours the alcohol is carefully drawn off and the precipitate washed with absolute alcohol. This is to be carefully done, and to avoid loss will require several days. The precipitate is then placed upon a thick paper filter and thoroughly washed by drawing alcohol through it by means of an exhaustion apparatus, after which the purified precipitate is collected and dried with care at a low temperature—best in a vacuum over sulphuric acid. A spongy, crumbling mass is thus obtained, which is easily crushed to form an extremely light white powder. This is readily soluble in water. It is not at all hygroscopic, and can be preserved in a dry condition without difficulty. The dose for a horse is 0.1 gramme.

De Schweinitz and Kilborne, in a paper published in November, 1892, state that in December, 1890, they

> "extracted from culture liquids of the Bacillus malleus an albumose which appeared to be the active principle in these cultures. At that time a preliminary experiment was conducted to see if this substance could be used to make guinea-pigs immune to the disease—glanders. The result was that out of a set of five, three vaccinated and two checks, only one, a vaccinated animal, recovered from an inoculation of a glanders culture. This experiment has since been repeated with sets of ten and twelve guinea-pigs each, with, at present writing, only negative results. A note of this work was published in the 'Annual Report of the Department of Agriculture for 1891.' The albumose was best obtained from the cultures, after the removal of the germ, by means of a Pasteur filter, by precipitation with absolute alcohol, resolution in water, and reprecipitation."

Babes (1892) claims to have succeeded in immunizing guinea-pigs against glanders by means of the toxic substances contained in cultures of the bacillus.

Foth (1894) has reported the results of extended experiments which have been made with his "Malleinum siccum" in Austro-Hungary. These results are stated as follows:

The experiments were for the most part made by Professor Schindelka, of Vienna. The tests were made with doses ranging from 0.1 gramme to 0.2 gramme. The number of horses treated, for diagnostic purposes, was four hundred and fifty-five; of these one hundred and forty-seven were examined post mortem. In general the infected horses reacted and the others did not. A reaction of 2° C. and upward, running a typical course, was evidence that the animal was infected, and such animals were killed and carefully examined by autopsy.

A reaction of 1.3° to 1.9° C., running a typical course, was taken as evidence that the animal was probably infected, and called for its isolation and a subsequent inoculation after an interval of four weeks.

A reaction of less than 1.2° C., or an atypical course of the febrile reaction, was taken as evidence of non-infection.

The typical febrile reaction consisted in a rapid or gradual elevation, according to the dose, then a fall of some tenths of a degree, a subsequent elevation to the highest previously reached point or above, and a gradual fall to the normal. The atypical reaction, which sometimes occurs in healthy animals, consists in an early and rapid elevation followed by an equally rapid fall to the normal. To properly distinguish the typical temperature curve, upon which the diagnosis depends, hourly observations are considered necessary.

Schütz (1894), as a result of his experiments on fifty-four horses, arrives at the conclusion that malleïn may give rise to the so-called "typical reaction" in horses which are not infected with glanders.

Hutyra and Preiz (1894), as a result of their extended researches, arrive at the conclusion that the use of malleïn constitutes the most important means for the early diagnosis of glanders in horses. They conclude that a temperature of 39.4° C. may be accepted as a safe positive malleïn reaction. According to them the reaction commences from four to six hours after the injection, and reaches its maximum in from eight to fourteen hours—rarely in sixteen to twenty hours. The return to the normal occurs in from twenty-four to thirty-six hours. The authors last named give the following directions for the preparation of malleïn: The virulence of the glanders bacillus is first increased by passing it through a series of guinea-pigs. Cultures are then made upon sterilized potato. When the culture and potato have become quite dry and dark colored they are collected in a glass dish and covered with a liquid consisting of equal parts of glycerin and distilled water, containing three to five parts per thousand of mercuric chloride. After standing for from ten to fourteen days in an incubating oven at 37.5° C., the liquid is filtered through paper and sterilized for an hour in a steam sterilizer. This liquid remains sterile on account of the presence of mercuric chloride, and may be preserved a long time without losing its activity. The dose is from 0.3 to 0.5 cubic centimetre, which is diluted to three cubic centimetres with carbolic acid water (0.5-per-cent solution). The diluted solution may also be kept a long time without losing its activity.

Bonome and Vivaldi (1892) have tested the action of malleïn obtained by precipitation with alcohol upon various animals. Guinea-pigs were found to resist comparatively large doses (ten to fifteen milligrammes), while rabbits and cats were more sensitive to the toxic action. In guinea-pigs and rabbits infected with glanders bacilli very small doses had a favorable influence upon the progress of the infection, and in healthy guinea-pigs a certain degree of immunity was induced by the repeated injection of small doses.

In a subsequent paper (1894) Bonome reports that he has had favorable results in the treatment of chronic glanders in man by doses of $\frac{1}{15}$ to $\frac{1}{20}$ cubic centimetre. The first dose is said to have caused an elevation of temperature, headache, polyuria, etc., but upon repeating the dose after two or three days a decided improvement of the general symptoms followed.

Chenot and Picq (1892) claim to have cured glanders in guinea-pigs by injections of blood serum from the ox, which animal has an immunity from the disease. They also state that the blood serum of the ox is germicidal for the glanders bacillus. Guinea-pigs treated with ox serum, either before or after infection, recovered in seven cases out of ten. When inoculated with very virulent cultures, which usually killed these animals in five days, the animals are said to have survived from twenty-one to forty-two days.

Bonome (1894) reports his success in curing infected guinea-pigs by means of filtered cultures made in the blood serum of the ox. He was not, however, successful in accomplishing this result with malleïn made in the usual way.

HOG CHOLERA.

The experiments thus far made with reference to protective inoculations against hog cholera have not given very satisfactory results. Selander and Metchnikoff have reported success in immunizing rabbits, but according to Smith their experiments were made with the bacillus of swine plague, and not with that of hog cholera as they supposed. The following conclusions have been formulated by Smith as a result of his extended experiments:

"1. It is possible to produce immunity toward hog-cholera and swine-plague bacteria in the very susceptible rabbit and the less susceptible guinea-pig. In the rabbit the only promising method of immunization toward hog cholera is the use of gradually augmented doses of attenuated cultures.

"2. Immunization toward swine plague is produced artificially with much greater ease than toward hog-cholera bacteria.

"3. The blood serum of animals protected against hog cholera and swine plague is almost as efficacious in producing immunity soon after treatment as the bacterial products obtained from cultures.

"4. Different degrees of culture in both hog cholera and swine plague lead to different forms of the inoculation disease. The greater the immunity short of complete protection the more prolonged and chronic the disease induced subsequently by inoculation.

"5. Pathogenic bacteria may remain in the organs of inoculated animals some time after apparently full recovery. Their presence may or may not be associated with lesions recognizable by the naked eye.

"6. The toxicity of sterilized cultures appears to be directly proportional to the number of bacteria in the injected fluid."

The experiments of Moore, reported in Bulletin No. 6 of the Bureau of Animal Industry, show that the bacillus of hog cholera does not become attenuated by being passed through rabbits, and that in the experiments of Metchnikoff, which led him to conclude that this is the case, the bacillus of swine plague, and not that of cholera, was used.

De Schweinitz studied the chemical products of the hog-cholera bacillus in 1890, and obtained from the cultures cadaverin, methylamine, a ptomaïne ("sucholotoxin"), and an albumose ("sucholoalbumin").

Novy (1890) has also obtained, by Brieger's method, a basic toxic substance ("susotoxin") which kills rats in the dose of 0.125 to 0.25 cubic centimetre. He also obtained from concentrated cultures, by precipitation with absolute alcohol, a toxalbumin which, when dried, killed rats in three or four hours in the dose of 0.05 to 0.01 gramme.

De Schweinitz in a later publication (1899) reports that he has obtained, by the method of Brieger and Boer for the isolation of the diphtheria antitoxin, an ash-free white powder, which possesses the antitoxic properties of serum from an immune animal; ninety cubic centimetres of serum gave him 0.152 gramme of this powder. The method referred to consists in precipitation by the use of zinc sulphate, repeated solution in sodium hydrate and precipitation by CO_2. In preparing serum for his experiments, cattle, horses, mules, and monkeys were employed. "The animals received injections of the filtered, sterile or live, cultures of the hog-cholera germ and swine-plague germ, respectively, or the solutions of their products, including cell contents, extracts, and secretions. These injections were made either subcutaneously, intravenously, or intra-abdominally, or a combination of two or more of these methods, depending upon the results obtained. The quantities given at first were small, but increased gradually until large amounts of the material used could be injected without bad results. This treatment of the animals must be carried out very carefully, and requires six to eight months' time before the serum is sufficiently potent to be of any practical use. As the treatment continues, the power of the serum to check the motility of the hog-cholera germ increases with rapidity. The length of immunity produced by the injection of serum is short, and more permanent immunity can apparently be secured by using in addition to serum the products of the germs."

The results of extensive inoculations (thirty-five thousand animals) which have been made by the Agricultural Department during the past two years have not yet been published, but it is understood that as a rule these results have been quite satisfactory.

HOG ERYSIPELAS.

Pasteur's first studies relating to the etiology of "*rouget*" were made, in collaboration with Chamberland, Roux, and Thuillier, in 1882. Pasteur found that the virulence of his cultures was increased by passing them through pigeons and diminished by passing them through rabbits. By a series of inoculations in rabbits he obtained an attenuated virus suitable for protective inoculations in swine. In practice he recommended the use of a mild virus first, and after an interval of twelve days of a stronger virus. These inoculations have been extensively practised in France, and the fact that immunity may be established in this way is well demonstrated. There has been some doubt, however, as to the practical value of the method, as its application has been attended with some loss, and there appears to be danger that the disease may be spread by the alvine discharges of inoculated animals. In a region where the annual losses from the disease are considerable, and where the soil is, perhaps, thoroughly infected with the bacilli, protective inoculations probably afford the best security against loss. But when it is practicable to stamp out the disease by quarantine of infected animals, disinfection of localities in which cases have occurred, and strict attention to cleanliness, this will probably be found the best method of combating the malady.

Chamberland (1894) states that in the preceding seven years, during which time protective inoculations were practised in France on a large scale, the mortality from rouget has been reduced to 1.45 per cent, whereas before these inoculations were practised the mortality from this disease was about twenty per cent. Losses amounting in some instances to as much as ten per cent have resulted from the inoculations. These are ascribed by Chamberland to secondary infection, through the inoculation wound, with other pathogenic bacteria.

Jakobi (1888) reports the results of inoculations made in 1887 and 1888 with "vaccines" obtained from Pasteur's agent in Paris. His results agree with those previously reported by Lydtin in showing a smaller loss, as a result of the inoculations, among young pigs than among older ones—over sixteen weeks. The loss among young pigs was only 1.3 per cent. The animals which survived subsequently escaped infection, while others not inoculated, associated with them, succumbed to the disease.

Hutyra has given the following statistics of inoculations made in Hungary during the year 1889, with "vaccines" obtained from the Pasteur laboratory in Vienna: 48,637 pigs were inoculated on 117 different farms. Of these 143 (0.29 per cent) died between the first and second inoculation. After the second inoculation 59 animals died (0.1 per cent). During the year following the inoculations, 1,082 inoculated pigs died of *Rothlauf*. Before the inoculations the annual loss in the same localities is said to have been from 10 to 30 per cent. Upon one farm 220 pigs which had been inoculated were associated with 1,500 not inoculated. The loss among the latter was 50 per cent; among the former 2.27 per cent.

In a later communication (1894) Jakobi gives the following results of inoculations made since by the same method: 1889, inoculated 133, loss 5; 1890, inoculated 151, loss 2; 1891, inoculated 158, loss 0; 1893, inoculated 223, loss 0; 1894, inoculated 145, loss 4. Total inoculated, 1,036; total loss, 14. These inoculations were made upon 19 different farms, and principally upon pigs less than four months old. The inoculated pigs were isolated to prevent the communication of the disease to other unprotected pigs.

Inoculations with Blood Serum of Immune Animals.—The experiments of Lorenz, commenced in 1891, seem to establish the fact that there is an antitoxin in the blood of animals which have an acquired immunity against this disease which may be used for producing immunity in other animals, or for the cure of the disease in animals already infected. In his latest communication (1894) Lorenz says:

> "When I read in the journals of the discovery of Behring and Kitasato that the blood of animals immunized against tetanus, when injected beneath the skin of other animals, gave them an immunity against tetanus, I had in my possession rabbits which were immunized against *Rothlauf*. I took from one of these some blood from the ear vein, injected it under the skin of a mouse,, inoculated this latter with a *Rothlauf* culture, and made the discovery, in this and a series of subsequent experiments, that the blood of an

animal immune against *Rothlauf* contains an immunizing substance. I further ascertained that this substance is found only in the blood serum, and not in the solid portions of the body organs, etc., and with the exception of the blood was found only in the secretions of serous membranes. I also found that the immunizing substance is only to be found for a certain time after renewed infection in the immune animals, and that it gradually disappears, without the loss of immunity in the animal, however. Finally, I discovered that the animals into which one injects blood serum from immune animals do not acquire a lasting immunity, but are only immune for a relatively short time."

In experiments made in 1893 and 1894, with a view to producing immunizing serum for protective inoculations on a large scale, Lorenz met with some disappointments; but he proposes to renew his attempts and hopes to avoid the difficulties which have been brought to light by experience, one of which he states as follows:

> "When an animal already immunized against *Rothlauf* receives an injection of a considerable quantity of a culture of the bacillus, in order to cause the production in its blood of a serum of high therapeutic value, the animal bears these injections without any notable reaction. But its blood serum contains during the following days, besides the immunizing substance, also poisonous substances, and blood which is taken too soon (twenty-four hours) after the injection has a toxic action upon animals which are already infected. If this poisonous serum is injected into a mouse which has been infected two days before with *Rothlauf* bacilli, in the dose of about 0.05 cubic centimetre, death occurs in a few hours, even when scarcely any evidence of sickness had been observed before the injection."

The fact that mice infected with this bacillus may be cured by injecting into them blood serum from an immunized rabbit has also been demonstrated by F. Klemperer (1892). In his experiments with the bacillus of mouse septicæmia, and with Friedländer's bacillus, he found that serum from an immune rabbit may be used to immunize mice and also to cure them after infection, while serum from a non-immune rabbit has no such action. The immunity produced in this way was found to be specific. That is, animals immunized against the pathogenic action of one of these bacilli were not protected against infection by the other. The "*Heilserum*" when added to cultures *in vitro* did not prove to have any special bactericidal action.

HYDROPHOBIA.

Notwithstanding the extended researches made, especially in Pasteur's laboratory, the etiology of hydrophobia still remains unsettled. It has been demonstrated by experiment that the virus of the disease is located in the brain, spinal marrow, and nerves of animals which have succumbed to the disease, as well as in the salivary secretions of rabid animals; and that the disease may be transmitted by intravenous inoculation, or by introducing a small quantity of virus beneath the dura mater, with greater certainty than by subcutaneous inoculations. But the exact nature of this virus has not been determined. The fact that a considerable interval elapses after inoculation before the first symptoms are developed indicates that there is a multiplication of the virus in the body of the infected animal; and this is further shown by the fact that after death the entire brain and spinal marrow of the animal have a virulence equal to that of the material with which it was inoculated in the first instance. The writer's experiments (1887) show that this virulence is neutralized by a temperature of 60° C., maintained for ten minutes—a temperature which is fatal to all known pathogenic bacteria in the absence of spores. But recent experiments show that certain toxic products of bacterial growth are destroyed by the same temperature. We are, therefore, not justified in assuming that the morbid phenomena are directly due to the presence of a living micro-organism; and, indeed, it seems probable, from what we already know, that the symptoms developed and the death of the animal are due to the action of a potent chemical poison of the class known as toxalbumins. But, if this is true, we have still to account for the production of the toxic albuminoid substance, and, in the present state of knowledge, have no other way to explain its increase in the body of the infected animal than the supposition that a specific, living germ is present in the virulent material, the introduction of which into the body of a susceptible animal gives rise to the morbid phenomena characterizing an attack of rabies.

Pasteur and his associates have thus far failed to demonstrate the presence of microörganisms in the virulent tissues of animals which have succumbed to an attack of rabies. Babes has obtained micrococci in cultures from the brain and spinal cord of rabid animals, and states in his article on hydrophobia in "Les Bacteries" (second edition, p. 791) that pure cultures of the second and third generation induced rabies in susceptible animals; but his own later researches do not appear to have established the etiological relation of this micrococcus.

Gibier (1884) has reported the presence of spherical refractive granules, resembling micrococci, in the brain of rabid animals, which he demonstrated by rubbing up a little of the cerebral substance with distilled water. As these supposed micrococci did not stain with the usual aniline colors and were not cultivated, it appears very doubtful whether the refractive granules seen were really microörganisms.

Fol (1885) claims to have demonstrated the presence of minute cocci, 0.2 μ in diameter, in sections of spinal cord from rabid animals, by Weigert's method of staining. The cords were hardened in a solution of bichromate of potash and sulphate of copper, colored with a solution of hæmatoxylon, and decolorized in a solution of ferrocyanide of potash and borax.

The writer (1887) has made similar preparations, carefully following the method as described by Fol, but was not able to demonstrate the presence of microörganisms in the numerous sections made. Nor have the observations of Fol been confirmed by the researches of other bacteriologists who have given their attention to the subject since the publication of his paper.

Pasteur first announced his success in reproducing rabies in susceptible animals by inoculations of material "from the medulla oblongata, the frontal lobes of the cerebral hemispheres, and the cerebro-spinal fluid" in a communication to the Academy of Sciences made on May 30th, 1881. At the same time he reported his success in the discovery of "a method for considerably shortening the period of incubation in rabies, and also of reproducing the disease with certainty." This was by inoculations made after trephining, upon the surface of the brain with material obtained from the brain of a rabid animal. Dogs inoculated in this way developed rabies in the course of two weeks, and died before the end of the third week—sometimes of furious rabies and sometimes of the paralytic form of the disease. In a second communication (December 11th, 1882) Pasteur reports his success in communicating the disease by the intravenous injection of virus from the central nervous system; also the experimental demonstration of the fact that all forms of rabies may be produced by the same virus; also that all portions of the spinal cord of rabid animals are virulent, as well as all parts of the brain; also that an animal (dog) which had recovered from a mild attack after inoculation proved to be subsequently immune, and that "this observation constitutes a first step toward the discovery of the prophylaxis of rabies." On February 25th, 1884, many important facts are stated which had been developed during the continuous study of the disease, and among others the fact that by passing the virus through a series of animals of the same species a fixed degree of virulence is established for each susceptible species, as shown by a definite and uniform period of incubation. By this method a virus had been obtained which produced rabies in rabbits in seven or eight days, and another which caused the development of the disease in guinea-pigs in five or six days after inoculation. In a subsequent communication (May 19th, 1884) evidence is given to show that by successive inoculations in monkeys the period of incubation is prolonged, and that the attenuated virus obtained from a monkey, after several successive inoculations in this animal, when inoculated into the dog, no longer produces fatal rabies; and that dogs so treated are subsequently immune.

In his address before the International Medical Congress at Copenhagen (August 11th, 1884), after a review of the facts developed during his experimental researches made during the preceding four years, Pasteur gives an account of the test made by a commission, appointed by the Minister of Public Instruction, to determine the efficacy of his method as applied to the protection of dogs. He says that he gave to the commission nineteen dogs which had been rendered refractory against rabies by preventive inoculations. These nineteen dogs and nineteen control animals, obtained from the pound without any selection, were tested at the same time. The test was made upon some of the animals of both series by inoculation with virulent material upon the surface of the brain, and upon others by allowing them to be bitten by rabid dogs, and upon still others by intravenous inoculations.

Not one of the protected animals developed rabies; on the other hand, three of the control dogs out of six bitten by a mad dog developed the disease, five out of seven which received intravenous inoculations died of rabies, and five which were trephined and inoculated on the surface of the brain died of the same disease. In a subsequent report the commission, of which M. Boulley was president, stated that twenty-three protected dogs which were bitten by ordinary mad dogs all remained in perfect health, while sixty-six per cent of the control animals, bitten in the same way, developed rabies within two months.

In his communication of October 26th, 1885, Pasteur reports his discovery of the fact that the virulence of the spinal cord of a rabbit is gradually attenuated by hanging it in a dry atmosphere, and is finally entirely lost; also that he had been able to make a practical application of this discovery in the protection of dogs by means of successive inoculations beneath the skin of an emulsion of spinal marrow attenuated in this way. The first inoculation was to be made with a portion of spinal cord which had been kept long enough to deprive it of all virulence, and this was followed by daily inoculations with more virulent material, until finally material was used from a cord only a day or two old.

With reference to his first inoculations in man, Pasteur says:

> "Making use of this method, I have already made fifty dogs of various races and ages immune to rabies, and had not met with a single failure, when, on the 6th of July, quite unexpectedly, three persons, residents of Alsace, presented themselves at my laboratory."

These persons were Theodore Vone, who had been bitten on the arm on July 4th; Joseph Meister, aged nine, bitten on the same day by the same rabid dog; and the mother of Meister, who had not been bitten. The child had been thrown down by the dog and bitten upon the hand, the legs, and the thighs, in all in fourteen different places. Pasteur commenced the treatment on July 6th, by injecting beneath the skin of this child an emulsion of cord which had been kept for fourteen days; this was followed by twelve more inoculations made on successive days with cord of increasing degrees of virulence—the last with cord a day old. On March 1st, 1886, Pasteur reported to the Academy of Sciences the fact that the boy Meister remained in good health and gave detailed information with reference to a number of cases which had since been treated by the same method.

With reference to the duration of the immunity resulting from these inoculations Pasteur says (1886) that out of fourteen dogs inoculated with "ordinary street virus," by trephining, at the expiration of a year after the protective inoculations had been practised, eleven resisted; out of six tested in the same way at the end of two years two proved to be immune.

In November, 1886, Pasteur communicated to the Academy of Sciences the results of his experiments with reference to a modification of his method as at first employed—the so-called intensive method. This modification consisted in making the inoculations with cords of increasing virulence in more rapid succession.

The method followed at Odessa, as reported by Gamaleía (1887), is shown below, the day being given above and age of the cord below.

1	2	3	4	5	6	7	8	9	10
14–13	12–11	10–9	8–7	6–5	4–3	2–10	8–6	4	2.

Since the adoption of this method and the use of larger quantities of virus, according to Gamaleía, there have been no deaths among those inoculated, numbering more than two hundred at the time the report was made. The author last referred to concludes from his experience that "the mortality diminishes in direct relation to the quantity of the vaccine injected."

Bujwid (1889) reports a total of 670 inoculations, with 9 deaths, made at Varsovie during the years 1886, 1887, and 1888. His method is shown below.

1	2	3	4	5	6	7
12–10	8–6	4	3	6	4	3.

The results of inoculations made at the Pasteur Institute in Paris during the years 1886 to 1890 are given in the following table:

Year.	Number Treated.	Died.	Mortality.
1886	2,671	25	0.94
1887	1,770	13	0.73
1888	1,622	9	0.55
1889	1,830	6	0.33
1890	1,540	5	0.32
Total	9,433	58	0.61

In the following table, A includes all persons treated who had been bitten by an animal proved to be rabid; B, persons bitten by animals examined by veterinary surgeons and pronounced rabid; C, persons bitten by animals suspected of being rabid. The figures relate to the year 1890:

	Number Treated.	Died.	Mortality.
A	416	0	....
B	909	4	0.44
C	215	1	0.46

Bordoni-Uffreduzzi gives the following statistics with reference to the inoculations practised at the Pasteur Institute in Turin during the years 1886 to 1891: 81 persons were inoculated by the method first proposed by Pasteur, with a mortality of 2.46 per cent; 925 persons were subsequently inoculated by the same method, but with larger doses of virus, with a mortality of 1.72 per cent. Finally, 338 persons were inoculated with still larger doses, with a mortality of 0.29 per cent.

At the Pasteur Institute in Palermo the number of persons inoculated in the four years prior to 1891 was 662, with a mortality among the inoculated of 0.6 per cent. In Bologna (1890) 210 persons bitten by dogs undoubtedly mad were inoculated, with a mortality of 0.47 per cent.

In the Pasteur Institute at Naples 810 persons were treated during the years 1886 to 1892, with a mortality of 0.86 per cent.

During the year 1891, 1,564 persons were inoculated at the Pasteur Institute in Paris, with a total mortality of 0.57 per cent. In 324 of these cases the animal which inflicted the bite was proved to be rabid by experimental inoculations.

Horsely (1889) has made a comparison of the results obtained by the "intensive treatment" as compared with those by the treatment first employed, and says:

"It is evident that the intensive treatment is very successful in coping with the worst cases, and that, instead of being itself a source of death, as asserted by those who gain notoriety and subsistence by villifying and misrepresenting scientific progress, it is a powerful agent in saving life."

The following table is given by Horsely "as showing the contrast between the old or simple treatment and the intensive treatment":

	Simple Treatment, 1886.	Intensive Treatment, 1888.
Odessa	3.39 per cent.	0.64 per cent.
Warsaw	4.1 "	0.0* "
Moscow	8.2† "	1.6 "

* The figures include sixteen months' work, and thirty individuals bitten in the face—four by wolves.

† This unusually high rate was found to be due to imperfections in the manner of preparing the cords for the inoculation material.

Perdrix (1890), in an analysis of the results obtained at the Pasteur Institute in Paris, calls attention to the fact that the mortality among those treated has diminished each year and ascribes this to improvement in the method. He says:

"At the outset it was difficult to know what formula to adopt for the treatment of each particular case. Upon consulting the accounts of the bites in persons who have died of hydrophobia notwithstanding the inoculations, we have arrived at a more precise determination as to the treatment suitable for each case, according to the gravity of the lesions. In the cases with serious wounds we inject larger quantities of the emulsion of cord and repeat the inoculations with the most virulent material. For the bites upon the head, which are especially dangerous, however slight their apparent gravity may be, the treatment is more rapid, and, above all, more intensive—that is to say, the virulent cord is injected several times."

The statistics arranged with reference to the location of the bite are given by Perdrix as follows:

Bitten upon the head, 684; died, 12 = 1.75 per cent.
" " ". hands, 4,396; " 9 = 0.2 "
" " " limbs, 2,839; " 5 = 0.17 "

Other methods of making susceptible animals immune against hydrophobia have been proposed and proved by experiment to be successful. Thus Galtier in 1880-1881 claimed that the sheep and the goat could be protected by intravenous injections of the virus of rabies, and more recent experiments fully confirm this. Protopopoff (1888) by injecting an emulsion of cord from a rabid animal into the circulation of dogs succeeded in protecting them from hydrophobia as a result of subsequent inoculation with virulent material upon the surface of the brain. He injected into a vein, at intervals of three days, one cubic centimetre of an emulsion of cord—first of six days, second of three days, third of one day. Roux had previously accomplished the same result by a single intravenous injection of a larger quantity (thirty-five cubic centimetres) of cord which had been kept for five or six days. In discussing his results Roux calls attention to the fact, which had been developed during his experiments, that the virulence of the spinal cord of rabid animals does not depend entirely upon the length of time it has been kept, but that large doses of cord kept as long as twelve days will sometimes produce hydrophobia when injected into the circulation of dogs, when smaller doses of cord kept five or six days prove to be inoffensive. He supposes that during desiccation the virus may not be equally acted upon throughout the cord, but that certain "islands" in the central portion may remain living and virulent when all the rest has been modified. A practical point with reference to the preservation of virulent material is referred to by Roux in a note published in the *Annals of the Pasteur Institute.* This is the fact that when preserved in glycerin, portions of the central nervous system retain their virulence for considerable time. Other forms of virus, *e.g.*, vaccine, may also be preserved in the same way.

Centanni (1892) has succeeded in making rabbits immune by inoculating them with an attenuated virus obtained by subjecting virulent material to the action of an artificial gastric juice. After digestion for less than twelve hours the virus still kills rabbits, when inoculated beneath the dura mater, but the period of incubation is considerably prolonged. After from twelve to twenty hours' digestion it no longer kills rabbits, but causes an infection, from which they recover, and after which they are immune.

Serum-therapy.—Tizzoni and Centanni (1892) have reported success in the treatment of infected rabbits by the use of blood serum from immune animals of the same species—immunized by the "Italian method" above described. The animals experimented upon were inoculated with a "street virus" which produced paralytic rabies in rabbits and caused their death in from fourteen to eighteen days. The blood serum was obtained from rabbits which had been proved to be immune by resisting inoculations of virus of full strength on the surface of the brain. The blood serum, in doses of three to five cubic centimetres, was injected subcutaneously, or into the peritoneal cavity, or into the circulation. Injections were made into each animal (in all from eleven to twenty-six cubic centimetres) after the first symptoms of paralytic rabies had appeared (on the seventh, the tenth, the eleventh, and the fourteenth day after infection). Four rabbits treated in this way fully recovered. In a subsequent experiment the bacteriologists named treated three rabbits with a dry antitoxin obtained by precipitation from the blood serum of immune rabbits. The precipitate was obtained by adding one part of serum to ten parts of alcohol, and was dried *in vacuo.* This dried precipitate, in doses of 0.18 to 0.25 gramme, was dissolved in sterilized water and injected as in the previous experiment. Commencing on the eighth day after infection five or six doses were given—in all 0.9 to 1.3 gramme. All of the animals treated recovered, while all of the control animals died. Babes had previously (1889) reported successful results in conferring immunity upon susceptible animals by injections of blood serum from immune animals.

Tizzoni and Schwartz, in pursuing this line of investigation (1892), report that while the blood serum of immune rabbits neutralizes the "fixed virus" of rabies *in vitro,* after short contact (five hours), the blood serum of immune dogs has but slight antitoxic potency. The immunizing substance in the rabbit serum does not dialyze, is soluble in glycerin, is precipitated by alcohol, and in general behaves like a globulin. In subsequent experiments Tizzoni and Schwartz used blood serum from dogs and rabbits immunized by Pasteur's method. The blood was drawn from the carotid of the immune animals, and the serum from the same, mixed with virulent spinal marrow in the form of a homogeneous emulsion, obtained by crushing and pressing through linen. These experiments corresponded with those previously made as to the superior antitoxic power of rabbit serum, which, after five hours' contact, neutralized the virulence of the emulsion of cord. By the injection of serum from an immune rabbit, in doses of five cubic centimetres, into the circulation of other rabbits, they were, as a rule, made immune. The immunizing substance (antitoxin) was shown by other experiments to be present only in the blood. Extracts from the liver, spleen, kidney, or muscles gave a negative result.

In a later communication (1894) Tizzoni and Centanni give an account of further experiments made principally upon sheep and dogs. By repeated inoculations they succeeded in obtaining from these animals a serum having an immunizing value of 1:25,000 or more, and from this a precipitate was obtained estimated to have a value of 1:300,000, and which in doses of 0.23 gramme (of the dried precipitate), dissolved in five times its weight of water, ought to be a sufficient dose to protect a man from the development of hydrophobia after being bitten by a rabid animal.

The authors named believe that inoculations with this antitoxin would be reliable for man, and that they would possess decided advantages over Pasteur's method of inoculation. These advantages are specified as follows:

"Applicability at any time during the period of incubation up to the moment of the appearance of symptoms of rabies; absolute absence of virulence and of any injurious action; very rapid treatment by the injection of one or several small doses of material; complete solubility and consequently prompt absorption of the material injected and its easy preservation in a dry condition."

INFLUENZA.

The bacillus discovered by Pfeiffer, in 1892, is now well established as the specific cause of this disease. Bruschettini has recently (1893) reported the details of his experiments upon rabbits, for which animals this bacillus is pathogenic. As a result of these experiments he has reached the following conclusions:

"1. Rabbits may be vaccinated against the pathogenic action of cultures of the influenza bacillus without great difficulty.

"2. The best material for producing a high grade of immunity is blood cultures which have been filtered through the Berkenfeld filter.

"3. The blood serum of immunized animals has strong antitoxic properties, but has no germicidal power.

"4. The serum of vaccinated animals has the power of conferring immunity upon other animals, in comparatively small amounts—in the proportion of 1:42,000 of body weight, and perhaps still less.

"5. This serum has also a decided curative action, and rescues rabbits from death even as late as forty-eight hours after infection by injection of a culture of the bacillus into the trachea."

These results lead the author to hope that serum-therapy may afford a method of curing this disease in man. For this purpose the blood of an immune rabbit would appear to be the most promising source from which to procure an antitoxic serum.

INFLUENZA IN HORSES.

SCHÜTZ (1887) has described a minute oval bacillus, usually associated in pairs, which appears to be the specific infectious agent in the disease known in Germany as *Brustseuche*. This bacillus is pathogenic for mice, rabbits, pigeons, and guinea-pigs, but not for swine or chickens. By injection of cultures into the parenchyma of the lungs Schütz reproduced the disease—confirmed in 1888 by Hell.

Horses which have suffered an attack of infectious influenza are subsequently immune, and the experiments of Hell have shown that an immunity also follows the disease which results from inoculations with pure cultures of the Schütz bacillus.

The extended experiments made by the War Department of the German Government show that the disease is not produced by intravenous injections or by the ingestion of the bacillus with the food. Infection occurs, however, when cultures are injected into the respiratory passages. Subcutaneous injections cause a painful local tumefaction, often followed by an abscess, but without the general symptoms of influenza.

Experiments have been made in Germany by Hell, Siedamgrotzki, and others, which indicate that the subcutaneous injection of blood serum from immune horses may confer immunity on other horses. Hell usually injected forty cubic centimetres at a time, and repeated this at intervals until two hundred to two hundred and forty cubic centimetres had been injected in the course of two or three weeks. He also reports the results of treatment by injections of blood serum into the trachea in horses already infected, and thinks these injections had a favorable influence on the course of the disease. Experiments made subsequently by Toepper have given a similar result, but others have not been so fortunate, and the immunizing value of blood-serum injections, as practised by the authors referred to, seems to be still a matter of some doubt. Toepper (1893) gives full directions for collecting the serum and a detailed account of results of experimental inoculations made by himself and others. He prefers to inject the serum into the breast over the ensiform cartilage. No reaction occurs after the injection.

PLEURO-PNEUMONIA OF CATTLE.

Protective inoculations against this disease have long been successfully practised. For this purpose serum obtained from the lungs of an animal recently dead has been employed, this having been proved by experiment to be infectious material, although the exact nature of the infectious agent present in it was not determined.

Willems, who was one of the first to advocate the use of protective inoculations in pleuro-pneumonia (1852), gave a lecture in 1894 in which he reviewed the evidence in favor of these inoculations in the disease under consideration. Various methods had been employed. Thus Willems states that the natives of the banks of the Zambéze cause animals to swallow a certain quantity of the liquid from the pleural cavity of an animal recently dead, and thus give them immunity. The virus has been injected into the circulation by some experimenters, and others have proposed to attenuate it by heat. But the method which has been most extensively employed is that discovered by the Dutch settlers at the Cape of Good Hope (the Boers), and consists in inoculating animals in the tail with serum from the lungs of an animal recently dead; or with a virus obtained from the tumefaction produced by such an inoculation in the tail.

This secondary virus was very extensively used by Lenglen, a veterinarian at Arras, who communicated his results to the Academy of Science at Paris, in April, 1863, and Willems says, in his last published communication, that this is the method which he prefers. It is also the method most extensively employed in Australia, into which country infectious pleuro-pneumonia was introduced in 1858. It quickly spread and has caused enormous losses. The killing of all animals, sick or suspected of being infected, was tried for several years; but this proved to be ineffectual for stamping out the disease, and the sacrifice was so great that this measure of prophylaxis was abandoned.

According to Loir, attention in Australia was called to Willems' method of protective inoculations, in 1861, by a letter from Cape Colony published in the journals of Sydney and in Melbourne. The method was at once applied both in Victoria and in New South Wales, and since that date many thousands of cattle have been inoculated. In order to obtain a sufficient supply of virus the method recommended by Pasteur in 1882 has been followed. This is described by Pasteur himself in the following words:

> "With a single lung we may procure sufficient virus to serve for numerous series of animals. And without having recourse to other lungs this provision may be maintained in the following manner: It is sufficient before the supply of virus is exhausted to inoculate a young calf in the dewlap or in the shoulder. The animal dies very promptly, and all its tissues near the point of inoculation are infiltrated with serum, which is virulent, and may be collected and preserved in a state of purity."

Loir prefers to obtain the virus in this way from a calf six to twelve months old, during the second week after inoculation, when the temperature of the animal has gone up to 40° to 42° C., as the virus is then said to possess the maximum degree of intensity. This vaccine seems to become attenuated in passing through a series of animals by inoculation, so that when it has been passed through a series of five animals it no longer produces death even when inoculated in the most dangerous localities. Loir testifies to the protective value of inoculations with this virus made in the tail of the animal, and gives the following example: A few months prior to the publication of his paper (1893), about two thousand cows were inoculated with a virus which had been passed through a series of five calves. At the moment of being driven away they were joined by nineteen other cows not vaccinated. After being on the road for a distance of two thousand kilometres, the animals arrived at their destination. The two thousand vaccinated were in good condition, while eight of the non-vaccinated had died of pleuro-pneumonia.

In the *Bulletin of the Central Society of Veterinary Medicine* of May 24th, 1894, M. Robcis reports the results of inoculations made with cultures of Arloing's *Pneumobacillus liquefaciens bovis,* and with injections of pulmonary serum. His statistics with reference to the last-mentioned "legal" inoculations he has obtained from official documents relating to the Department of the Seine.

The total number of infected localities in this department during the years 1885 to 1891 was 1,253; total number of contaminated animals, 18,356; total number inoculated, 18,359; total number of deaths prior to inoculation, 1,753; total number of deaths after inoculation, 2,741; total number of deaths due to the inoculation, 94; total percentage of mortality, 22.8 per cent. After discussing these and other statistics Robcis arrives at the conclusion that Arloing's method of preventive inoculations with cultures of the *Pneumobacillus liquefaciens bovis* gives better results than the legal method with serum from an infected animal, the total loss among animals exposed to contagion not being over twelve to fourteen per cent.

Nocard (1892) says that serum from the lungs of an animal dead from pleuro-pneumonia preserves its virulence and usefulness as a vaccine, when mixed with half a volume of pure neutral glycerin and half a volume of a five-per-cent solution of carbolic acid. At the end of two and a half months this mixture preserved its full virulence.

PNEUMONIA.

The micrococcus of croupous pneumonia was discovered by the present writer in the blood of rabbits inoculated subcutaneously with his own saliva in September, 1880. In 1885 this micrococcus, which I had repeatedly obtained in pure cultures from the blood of rabbits inoculated, as in the first instance, with my own saliva, was identified with the micrococcus of the same form present in the rusty sputum of patients with pneumonia. In a paper read before the Pathological Society of Philadelphia, in April, 1885, and published in the *American Journal of Medical Sciences* on July 1st of the same year, I say:

> "It seems probable that this micrococcus is concerned in the etiology of croupous pneumonia, and that the infectious nature of the disease is due to its presence in the fibrinous exudate into the pulmonary alveoli."

This has since been fully established by the researches of Fränkel, Weichselbaum, Netter, Gameleía, and many others. Fränkel first discovered this micrococcus in his own salivary secretions in 1883, and his first paper relating to its presence in the exudate of croupous pneumonia was published on July 13th, 1885, *i.e.*, thirteen days after the publication of the paper from which the above quotation is made. Under these circumstances the writer feels justified in again calling attention to his priority in the discovery of this important pathogenic micrococcus, and in objecting to its being described as "Fränkel's pneumococcus," the "diplococcus of Fränkel," etc.

In my paper above referred to (July, 1885) I described this micrococcus under the name of Micrococcus Pasteuri, but in my "Manual of Bacteriology" (1892) it is described under the name of *Micrococcus pneumoniæ crouposæ.*

This micrococcus is very pathogenic for mice and for rabbits, less so for guinea-pigs and for dogs. Like other pathogenic microörganisms of the same class, it varies greatly in virulence when obtained from different sources. In the saliva of healthy persons, which seems to be its normal habitat, it sometimes has comparatively little virulence. On the other hand, when contained in the blood or in an exudate from a serous cavity of an infected rabbit or mouse, it is very virulent. In one instance (1881) the writer has seen a fatal result in a dog from the subcutaneous injection of one cubic centimetre of bloody serum from the subcutaneous connective tissue of a rabbit recently dead.

Pneumonia never results from subcutaneous injections into susceptible animals, but injections through the thoracic walls into the lung may induce a typical fibrinous pneumonia. This was first demonstrated by Talamon (1883), who injected the fibrinous exudate of croupous pneumonia, obtained after death, or drawn during life by means of a Pravaz syringe, from the hepatized portion of the lung, into the lungs of rabbits. Gameleía has also induced pneumonia in a large number of rabbits, and also in dogs and sheep, by injections directly into the pulmonary tissue. Sheep were found to survive subcutaneous inoculations, unless very large doses (five cubic centimetres) of a virulent culture were injected. But intrapulmonary inoculations are said to have invariably produced a typical fibrinous pneumonia which usually proved fatal. In dogs similar injections gave rise to a "frank, fibrinous pneumonia which rarely proved fatal, recovery usually occurring in from ten to fifteen days, after the animal had passed through the stages of red and gray hepatization characteristic of this affection in man."

Without doubt an attack of pneumonia is followed by a certain degree of immunity of longer or shorter duration. According to Ruge, who has made a careful study of the subject, relapses are very infrequent—indicating a temporary immunity—but subsequent attacks are more likely to occur in those who have once suffered an attack of the disease, and as many as four or five attacks have been known to occur in the same individual.

In 1,100 cases collected by Wagner but 2 relapses occurred (= 0.18 per cent). Ruge reports that in 440 cases treated at the Charité in Berlin there were but 2 relapses. The liability to subsequent attacks at a later period is shown by the following figures, which we copy from Ruge's paper: In 280 cases reported by Stortz, 26.4 per cent had previously suffered an attack of the disease; in 133 cases reported by Morhart the proportion of previous attacks was 41.3 per cent; in 157 cases by Pohlmann, 34.4 per cent; in 166 cases by Schapira, 31.3 per cent; in 128 cases by Keller, 36.9 per cent; in 175 cases by Grisolle, 30.9 per cent.

The writer, in a series of experiments made during the winter of 1880-81, obtained experimental evidence which showed that susceptible animals (rabbits) acquire immunity from the pathogenic action of this micrococcus as a result of inoculations with an attenuated virus. The experiments referred to had as their object the determination of the comparative value of various germicidal agents, as tested upon this micrococcus; incidentally it was found "that a protective influence has been shown to result from the injection" (into rabbits) "of virus, the virulence of which has been modified, without being entirely destroyed, by the agent used as a disinfectant." (Quoted from the writer's report of the experiments referred to, "Studies from Biological Laboratory," Johns Hopkins University, Baltimore, 1882.)

In 1891 G. and F. Klemperer published an important memoir relating to the pathogenic action of this micrococcus and the production of immunity in susceptible animals by means of filtered cultures. In some cases this immunity was found to last as long as six months. A curious fact developed in their researches was that the potency of the substance contained in the filtered cultures was increased by subjecting these to a temperature of 41° to 42° C. for three or four days, or to a higher temperature (60° C.) for an hour or two. When injected into a vein after being subjected to such a temperature, immunity was complete at the end of three or four days; but the same material, not so heated, required larger doses and a considerably longer time (fourteen days) to confer immunity upon a susceptible animal. The unwarmed material caused a considerable elevation of temperature, lasting for some days. The authors mentioned conclude from their investigations that the toxic substance present in cultures of Micrococcus pneumoniæ crouposæ is a proteid substance, which they propose to call pneumotoxin. The substance produced in the body of an immune animal, as a result of protective inoculations, upon which the immunity of these animals depends, is also a proteid, which they call antipneumotoxin. This they isolated from the blood serum of immune animals. By experiment they were able to demonstrate that the blood serum containing this protective proteid, when injected into other animals, rendered them immune; and also that it arrested the progress of the infectious malady induced by inoculating susceptible animals with virulent cultures of the micrococcus. When injected into the circulation of an infected animal, its curative action was manifested by a considerable reduction of the body temperature. The toxalbumin was obtained from filtered bouillon cultures of a virulent variety of the micrococcus of pneumonia, in the form of an amorphous, yellowish-white powder. This was thrown down from the filtered cultures by means of alcohol, and again dissolved in water and reprecipitated in order to purify it.

Issaeff (1893) as a result of his experiments has found that the virulence of this micrococcus can be greatly increased by successive inoculations in the peritoneal cavity of rabbits, and that after a series of ten or twelve such inoculations the blood of the infected animal does not coagulate and becomes extremely toxic. In order to obtain the toxins from this blood, Issaeff collects the blood of three or four animals just dead in a sterilized vessel, and adds to this an equal volume of sterilized water containing one per cent of glycerin, made alkaline by the addition of a few drops of a concentrated solution of bicarbonate of soda. The mixture is sterilized by passing it through a Chamberland filter. This liquid sometimes kills rabbits when injected into the circulation in the proportion of one per cent of the weight of the animal. When heated to 70° C. its toxic power is considerably diminished, and a temperature of 100° C. neutralizes it completely.

Emmerich (1891) has succeeded in immunizing rabbits and mice by the intravenous injection of a very much diluted but virulent culture of the micrococcus. Other rabbits and mice were rendered immune by injecting into them material obtained from rabbits immunized with diluted cultures. The flesh of these animals was rubbed up into a pulp, and the juices were obtained by pressure through a piece of sterilized cloth. The bloody juice, after standing for twelve hours at a temperature of 10° C., was passed through a Pasteur filter and then served to immunize the animals referred to.

Belfanti (1892) has succeeded in immunizing rabbits against the pathogenic action of this micrococcus by injecting into the circulation a filtrate obtained from the sputa of pneumonia cases. The viscid sputa mixed with an equal part of distilled water was kept on ice for twenty-four hours and then passed through a Chamberland filter. Ten cubic centimetres of this filtrate was injected into the ear vein of rabbits. Some of the animals so treated proved to be immune against general infection when inoculated with a virulent culture of the micrococcus, but they had a localized inflammation and œdema about the point of inoculation. After recovering from this they proved to be entirely refractory against subsequent inoculations.

Foà and Scabia (1892) have reported success in producing immunity with filtered cultures, and also with a glycerin extract from the blood of an infected rabbit. This, after filtration, was injected subcutaneously in doses of two cubic centimetres at intervals of five days. The authors named have also produced immunity in rabbits by the use of "pneumo-protein." This is an extract from the bacterial cells obtained by first collecting these from the surface of a Chamberland filter through which the cultures have been passed; then digesting them for three hours at 55° C. in a five-per-cent solution of glycerin. According to Foà and Scabia immunity produced in this way is more decided and of longer duration than that resulting from the other methods tested by them.

Mosny (1892) has also made numerous experiments which show that rabbits may be immunized by means of filtered cultures, or by the juices from the tissues of an immune animal obtained by maceration and filtration. When sterilized cultures were employed the best results were obtained by first heating very virulent cultures for three hours at 60° C. The dose employed was ten cubic centimetres, and immunity was not established immediately but required a period of at least four days for its development.

The blood serum of immune rabbits was not found to have any bactericidal power, and the micrococcus of pneumonia preserved its vitality longer in the blood serum of immune rabbits than in that of other animals of the same species.

G. and F. Klemperer had previously reported that the blood of immune rabbits does not destroy the micrococcus of pneumonia or restrict its development.

Issaeff (1893) also reports his success in immunizing rabbits by means of sterilized cultures or filtered blood from infected animals recently dead. A single intravenous injection of ten cubic centimetres of filtered blood, prepared as heretofore indicated (p. 340), sufficed to confer immunity. To test immunity the animals were subsequently inoculated with two to four drops of virulent blood; and to maintain it the inoculations (0.5 cubic centimetre) were repeated every four weeks. Although immune against infection these animals are said not to have acquired any immunity against the toxins of the micrococcus of pneumonia. Contrary to the conclusion reached by G. and F. Klemperer, Issaeff concludes from his experiments that "rabbits, although completely refractory against pneumonic infection, remain highly sensitive to the toxins of this microbe. Even small doses of the toxins are not neutralized in the blood of vaccinated animals. We are therefore brought to the conclusion that the existence of an antitoxic property of the blood of vaccinated animals cannot be admitted."

The serum of immunized rabbits was not found by Issaeff to possess any bactericidal power for the micrococcus of pneumonia, and no attenuation of virulence occurred as a result of cultivation in this serum. But when introduced beneath the skin of an immune rabbit, the micrococcus quickly loses its virulence. At the end of eighteen hours it has completely lost its pathogenic power, and cultures made in bouillon no longer have any injurious effect upon rabbits. This attenuating effect produced in the body of an immune animal is ascribed by Issaeff to the action of phagocytes, which are said to be very numerous, and in the course of five or six hours to pick up all of the cocci in the vicinity of the point of inoculation. These are not, however, immediately destroyed in the interior of the phagocytes, but preserve their vitality for nearly forty-eight hours, and when introduced into bouillon give a culture which has no longer any pathogenic virulence.

RINDERPEST.

The disease of cattle known in Germany as rinderpest is due to a bacillus closely resembling the bacillus of fowl cholera and of swine plague (*Bacillus septicæmiæ hæmorrhagicæ*).

Professor Semmer, of St. Petersburg, has reported (1892) his success in immunizing cattle against this disease. The virulence of cultures was attenuated by passing them through guinea-pigs, or by exposure to heat, and this attenuated virus was used in protective inoculations. Semmer says:

> "By the subcutaneous injection of blood serum from immune animals their susceptibility to rinderpest was diminished, and such blood serum destroyed the 'rinderpest contagium' in one to twenty-four hours."

SWINE PLAGUE.

As stated in the chapter on cholera in fowls, the bacillus of swine plague (*Schweineseuche*, Löffler and Schütz) very closely resembles Pasteur's microbe of fowl cholera and Koch's bacillus of rabbit septicæmia, and if not identical with these at least varies from them so slightly in its morphological and biological characters that recent authors do not feel justified in considering it a distinct species. Koch first obtained his bacillus of rabbit septicæmia by inoculating rabbits with putrefying flesh infusion. Gaffky produced the same infectious disease in rabbits by inoculating them with impure river water. Davaine had previously obtained similar results by inoculating rabbits with putrefying blood. The writer in 1887 produced the same disease in rabbits, while in Cuba, by inoculating them with putrefying liver from a yellow-fever cadaver. A similar, and possibly identical, bacillus has been found in the blood of deer (Hueppe), of cattle (Kitt, and of buffalo (Oreste-Armanni) suffering from a fatal infectious disease. And all of these allied species or varieties are included by Hueppe and by the present writer under the single specific name *Bacillus septicæmiæ hæmorrhagicæ.* The bacillus of the disease known in this country as swine plague, according to Smith, agrees in all particulars with that of the German swine plague (*Schweineseuche*) described by Löffler and Schütz, except that the latter is more pathogenic for swine and for rabbits.

In a publication by Smith and Moore (United States Department of Agriculture, Bureau of Animal Industry, Bulletin No. 6, 1894) they have given an account of their experiments relating to immunizing animals against the pathogenic action of this bacillus. The bacilli used in these experiments were sufficiently virulent to kill rabbits in twenty hours when injected beneath the skin of these animals in doses of 0.001 cubic centimetre of a fresh bouillon culture. The experiments were made upon young adult rabbits by various methods, viz.: with sterilized bouillon cultures; with sterilized suspensions of agar cultures; with the filtrate of agar suspensions; with defibrinated, sterilized blood of infected rabbits; with blood serum from immune animals.

> "A greater or less degree of immunity was produced in rabbits by sterilized bouillon cultures, sterilized agar suspensions, sterilized blood from infected rabbits, and blood serum from immunized rabbits. The sterilized blood of diseased rabbits was capable of producing immunity, while the blood serum of immune rabbits produced rather equivocal results."

The different degrees of immunity which may be acquired by rabbits, as shown by a subsequent inoculation with virulent material, are classified by Moore as follows:

> "1. No resistance—acute septicæmia.
> "2. Slight resistance—peritonitis.
> "3. Increased resistance—pleuritis and pericarditis with or without secondary pneumonia.
> "4. Higher degree of resistance—pleuritis and peritonitis.

"5. Still greater resistance—irregular lesions in the form of abscesses, subcutaneous and subperitoneal.

"6. Nearly complete immunity—very slight reaction at the point of inoculation."

Up to the year 1894 the bacteriological experts of the Department of Agriculture had not proposed to make a practical application of the facts developed in their experimental work in the way of protecting herds of swine by means of inoculations with an attenuated virus, or with sterilized cultures. In the report on swine plague, made by the Bureau of Animal Industry published in 1891, the following measures for arresting an epidemic are recommended:

"When the disease has actually appeared in a herd the question generally arises whether it is worth while to make any attempt to save a portion of the herd or to leave them to their fate. As a rule it may be stated that it is best to slaughter both healthy and diseased at once, and give the surroundings sufficient time to rid themselves of the infection before fresh animals are brought into them. If this be not desirable, we should recommend the following measures to be vigorously carried out:

"*a.* Removal of still healthy animals to uninfected grounds or pens as soon as possible.

"*b.* Destruction of all diseased animals.

"*c.* Careful burial or burning of carcasses.

"*d.* Repeated thorough disinfection of the infected premises.

"*e.* Great cleanliness both as to surroundings and as regards food."

In the same report (1891) the following reference is made to protective inoculations:

"As regards swine plague the experiments which have thus far been carried out indicate that this disease may prove amenable to preventive inoculation. We have been able, by the injection of both living cultures and those sterilized at a low temperature (58° C.), to make the most susceptible animals —rabbits—insusceptible to the most virulent swine plague bacteria. By two subcutaneous injections of cultures of swine-plague bacteria, swine have been made insusceptible to doses injected into the circulation which proved fatal to control pigs within twenty-four hours."

According to Smith the experiments of Metchnikoff (1892), reported as made with the bacillus of hog cholera, were in fact made with the bacillus of swine plague; we therefore refer to them here. These experiments showed that rabbits could be easily immunized against the pathogenic action of virulent cultures by means of blood, from an infected animal, sterilized by heat. Doses of 1.5 cubic centimetres, or more, were fatal to rabbits; but smaller doses, repeated several times, given either subcutaneously or by injection into the circulation, caused the animal to become immune.

STREPTOCOCCUS INFECTION.

It is now generally recognized by pathologists that erysipelas, puerperal fever, certain forms of diphtheritic inflammation of the fauces, and certain acute abscesses are due to infection by a streptococcus described by recent authors under the name of *Streptococcus pyogenes.* This streptococcus, like other pathogenic microörganisms of the same class, varies greatly in its pathogenic power as a result of conditions relating to the source of the particular variety under cultivation. As obtained from a case of erysipelas or puerperal fever it is extremely virulent, but when it has led a saprophytic existence for some time, or has been cultivated for a considerable time in the usual artificial culture media, its pathogenic potency is greatly diminished.

Mironoff (1893) has made a series of experiments with a view to determining whether rabbits can be immunized against the pathogenic action of this streptococcus, and has obtained successful results by the following method:

Vigorous rabbits, weighing two kilogrammes, were inoculated subcutaneously with from three to six cubic centimetres of a sterilized bouillon culture of the streptococcus. Cultures three days old were employed, and these were sterilized for twenty minutes at 120° C.—the reason for using so high a temperature is not apparent, inasmuch as this streptococcus is destroyed in a few minutes by a temperature of 60° C. At the end of ten to fifteen days, "when the animal has fully recovered," a second dose of from six to twelve cubic centimetres of a culture, sterilized in the same way, is injected beneath the skin. After another interval of ten to fifteen days two cubic centimetres of a virulent non-sterilized culture are injected subcutaneously, and this is repeated with gradually increasing doses (one to two cubic centimetres more) at intervals of the same period. Finally the animals "support without reaction" a dose five times as great as would be required to kill an animal of the same weight not immunized. But the author adds that more than half the animals thus treated died before the completion of the immunizing process. These deaths resulted from local infectious processes, such as peritonitis, pericarditis, meningitis, or abscesses formed at the point of inoculation.

Further experiments showed that the blood serum of animals immunized in this way when injected into susceptible animals (rabbits) in the dose of 1.5 cubic centimetres per kilogramme of body weight conferred upon them a certain degree of immunity against streptococcus infection, and with twice this amount (three cubic centimetres) a very decided immunity was produced. The blood serum of immune rabbits in doses of three to four cubic centimetres per kilogramme of body weight was found to exercise a curative power, and completely to arrest the acute septicæmia resulting from inoculations with a virulent culture of this streptococcus, or to cause the disease to run a chronic course, with formation of abscesses and final recovery.

In this connection we may call attention to the experiments of Emmerich (1886), which show that the fatal course of anthrax infection, in rabbits, may be arrested by the subcutaneous or intravenous injection of this streptococcus. Subsequent experiments by Emmerich and de Mattei (1887) showed that eleven hours after such an injection the anthrax bacilli were all dead and were already undergoing degenerative changes.

Emmerich and his associates (1894) have reported numerous additional experiments which show that the blood serum of a rabbit which is suffering from streptococcus septicæmia (third day), when filtered through a Pasteur-Chamberland filter to remove all living cocci, may be used with success in arresting anthrax infection in rabbits. The filtered serum was given four hours after the anthrax infection in the dose of twenty-five cubic centimetres in the peritoneal cavity and fifteen cubic centimetres subcutaneously. This was repeated the following day at nine o'clock in the morning and five o'clock in the evening, and again on the third day in the morning. Favorable results were also obtained by using in the same way blood serum from a sheep infected with the streptococcus.

Cobbett (1894) reports success in immunizing rabbits by means of attenuated varieties of the streptococcus or by filtered cultures. Also that cutaneous erysipelas, produced by inoculation, after recovery leaves the patient immune from a repetition of the local inflammatory process as a result of a subsequent inoculation, and also confers a general immunity against streptococcus infection. But this immunity is of short duration, not lasting longer than a few weeks. Inoculation in the ear of a rabbit, protected by a previous inoculation in the same locality, is followed by an inflammatory reaction; but this is of brief duration and has disappeared before the erysipelatous inflammation produced in a control is well under way.

SYMPTOMATIC ANTHRAX.

This disease of cattle is popularly known as "black leg," or "quarter evil," and is described by German authors under the name of *Rauschbrand*—French, "*charbon symptomatique.*" The disease prevails during the summer months in various parts of Europe, and to some extent in the United States. It is characterized by the appearance of irregular, emphysematous swellings of the subcutaneous tissues and muscles, especially over the quarters. The muscles in the affected areas have a dark color and contain a bloody serum in which the bacillus is found to which the disease is due. This is an anaërobic bacillus which forms large oval spores.

The etiology of the disease was first clearly established by the researches of Arloing, Cornevin, and Thomas (1880 to 1883), and subsequent researches have shown that immunity may be produced in susceptible animals by protective inoculations.

The disease causes considerable losses among cattle in certain sections. Horses do not contract it spontaneously, and when inoculated with a culture of the bacillus present only a limited local reaction. Swine, dogs, rabbits, fowls, and pigeons have but slight susceptibility. The researches of the authors above mentioned have shown that the virulence of a culture is greatly increased by adding to it twenty per cent of lactic acid. The guinea-pig is the most susceptible animal, and succumbs in from twenty-four to thirty-six hours when inoculated subcutaneously with a small quantity of a pure culture. According to Kitasato cultures in a bouillon made from the flesh of the guinea-pig soon lose their virulence, while cultures in solid media preserve their virulence for a long time. Cultures are readily attenuated by heat, according to the method of Toussaint and Chauveau—exposure to a temperature of 42° to 43° C. in the absence of spores. The spores are attenuated by exposure for several hours to a temperature of 80° to 100° C. Arloing, Cornevin, and Thomas recommend for the production of immunity in cattle inoculation with a dried powder of the muscles of animals recently dead from the disease. This is attenuated by heat. According to Kitt the muscles should first be dried at 32° to 35° C. and then powdered. Two "vaccines" are prepared from this powder—a strong vaccine by exposure to a temperature of 85° to 90° C. for six hours, and a weaker vaccine by exposure for the same time to a temperature of 100° to 104° C. (dry heat). An inoculation is first made with the weaker vaccine which gives rise to a local reaction of moderate intensity. Later a second inoculation is made with the stronger vaccine, after which the animal is immune from the pathogenic action of the most virulent material. Immunity may also be secured by intravenous injections; or, in guinea-pigs, by inoculations with cultures which have become attenuated by being kept a few days, or by exposure to a temperature of 42° to 43° C.; or by inoculation with a very small quantity of a pure culture; or by inoculations with filtered cultures (Roux and Chamberland); or with cultures sterilized by heat (Kitasato). A non-fatal and protective local infection may also be produced in cattle by inoculations with virulent material made into the extremity of the tail. Roux has claimed that animals which have an acquired immunity against symptomatic anthrax are also immune against the pathogenic action of the bacillus of malignant œdema; but Kitasato was unable to confirm this.

Strebel, in 1885, published the results of protective inoculations made in Switzerland in 1884. The inoculations were made in the end of the tail with two "vaccines," with an interval between the two of from nine to fourteen days. The vaccines were prepared by exposure to heat, as above recommended by Arloing, Cornevin, and Thomas. The most favorable season for inoculations was found to be the spring, and the most favorable age of cattle for inoculation from five months to two years.

In seven Swiss cantons 2,199 cattle were inoculated; 1,810 inoculations were made among animals which were exposed in dangerously infected pastures. Of these but 2 died, one two months and the other four months after the protective inoculations. Among 908 inoculated cattle, which were pastured with 1,650 others not inoculated, the mortality was 0.22 per cent, while the loss among the latter was 6.1 per cent. The following year (1885), according to Strebel, the number of inoculations, exclusive of those made in the canton of Bern, was 35,000. The losses among inoculated animals are reported as having been about five times less than among those not protected in this way. In the canton of Bern, in the same year, according to Hess, 15,137 cattle were inoculated by thirty-eight veterinarians—12,190 of these were pastured in dangerously infected pastures. The results are said to have been favorable to the method, but the abstract at hand does not give the precise figures.

In 1887 Kitt reported the results of his investigations, which were confirmatory of those previously published by Arloing, Cornevin, and Thomas, and also of a new method of inoculation, which presented the advantage that a single inoculation was sufficient to confer immunity. This was made in the region of the shoulder with a vaccine somewhat stronger than that employed by the French bacteriologists, but which was found to be without danger for cattle. It produced only a slight local effect. His vaccine was prepared by heating the moistened flesh of an animal just dead from the disease to 85° to 90° C. for six hours. This did not kill the spores present, but caused a sufficient attenuation in their virulence.

In a later communication (1888) Kitt recommends that the flesh of the diseased animal be first dried and pulverized, and then subjected to a temperature of 100° C. in streaming steam for six hours, after which it is to be again dried and used for subcutaneous inoculations. The dose is from five to fifteen centigrammes.

Roux (1888) has shown by experiment that sterilized cultures of the bacillus, which have been exposed to a temperature of 115° C., when injected in doses of forty cubic centimetres, three times repeated, into the cavity of the abdomen of guinea-pigs, cause these animals to be completely immune against the most virulent material. Cultures from which the bacilli have been separated by filtration are still more active. And immunity could easily be conferred by the subcutaneous inoculation, in guinea-pigs, of one cubic centimetre of the filtrate from the serum obtained from the œdematous tissues of a diseased animal.

Schuhanka (1888) has reported the results of inoculations made in the dukedom of Salzburg during the year 1887. In all 2,596 cattle were inoculated once, and 2,472 twice, with an attenuated virus, in forty-seven different parishes. Most of these were from six months to a year old. No losses occurred as a result of the inoculations. During the summer of 1887 the 2,472 cattle which had been twice inoculated were associated in infected pastures with 3,561 unprotected cattle. The loss among the former was 8, = 0.32 per cent; among the latter it was 235, = 6.31 per cent.

Strebel reports similar results, in 1887, in the canton Freiburg, where 1,725 cattle which had been inoculated suffered a loss of 0.23 per cent, and 1,945 associated cattle a loss of 5.28 per cent.

Lydtin (1892) reports the results of inoculations made in five districts (*Amtsbezirken*) in Baden during the years 1886–91: 2,797 cattle were inoculated with a loss of 3 only as a result of the inoculation. None of the inoculated cattle subsequently contracted the disease.

In the *Bulletin of the Central Society of Veterinary Medicine* of France (1892), Guillod and Simon give the results of 3,500 inoculations made since 1884. The mortality among cattle in the region where these inoculations were practised had been from 10 to 20 per cent, but fell to 0.5 per cent among the inoculated animals.

The authors last named prefer inoculations in the region of the shoulder to the plan first practised of inoculating in the end of the tail. Strebel also (1892) advocates this method, which is quickly carried out and attended with but little loss. According to Strebel the loss among 13,022 inoculated in this way only amounted to 5, while the loss among animals inoculated by the old method was twice as great.

TETANUS.

The experiments of Kitasato (1889) show that pure cultures of the tetanus bacillus injected into mice, rabbits, or guinea-pigs produce typical tetanic symptoms and death. As the presence of this bacillus at the seat of injury, in cases of tetanus in man, has now been demonstrated by numerous observers, there is no longer any question that tetanus must be included among the traumatic infectious diseases, and that the bacillus of Nicolaier and of Kitasato is the specific infectious agent. Kitasato's experiments (1890) show that cultures of the tetanus bacillus which have been sterilized by filtration through porcelain produce the same symptoms, and death, in the animals mentioned, as result from inoculation with cultures containing the bacillus. It is evident, therefore, that death results from the action of a toxic substance produced by the bacillus. This is further shown by the fact that the bacillus itself cannot be obtained in cultures from the blood or organs of an animal which has succumbed to an experimental inoculation with an unfiltered culture; but the blood of an animal killed by such an inoculation contains the tetanus poison, and when injected into a mouse causes its death with tetanic symptoms.

When a platinum needle is dipped into a pure culture of the tetanus bacillus, and a mouse is inoculated with it subcutaneously, the animal invariably falls sick within twenty-four hours and dies of typical tetanus in two or three days. Rats, guinea-pigs, and rabbits are killed in the same way by somewhat larger quantities—0.3 to 0.5 cubic centimetre (Kitasato). Pigeons are very slightly susceptible. The tetanic symptoms are first developed in the vicinity of the point of inoculation: if the animal is inoculated in the posterior portion of the body, the hind legs first show tetanic contraction; if in the forepart of the body, the muscles of the neck are first affected. At the autopsy there is a certain amount of hyperæmia at the point of inoculation, but no pus is formed; in inoculations with garden earth, or accidental inoculations in man, pus is commonly found in the vicinity of the inoculation wound. The various organs are normal in appearance. Kitasato says that he has not been able to demonstrate the presence of the bacillus or of spores in the spinal marrow, the nerves, muscles, spleen, liver, lungs, kidneys, or blood from the heart; nor has he been able to obtain cultures from the various organs. In mice which were inoculated at the root of the tail Kitasato was able to demonstrate the presence of the bacilli at the point of inoculation by the microscopical examination of an excised piece of the tissues for eight to ten hours after the inoculation; later than this they were not found. In pus from the inoculation wounds of men and animals accidentally infected the bacilli are present, but the formation of spores does not always occur. According to Kitasato, the sooner death has occurred after accidental inoculation the less likely are spores to be found in the rods, but from pus in which no spores are seen cultures of the bacillus may be obtained in which spores will develop in the usual manner.

Guinea-pigs are even more susceptible to the tetanus poison than mice, and rabbits less so. The amount of filtrate from a slightly alkaline bouillon culture required to kill a mouse is extremely minute —0.00001 cubic centimetre (Kitasato). The tetanic symptoms are developed within three days; if the animal is not affected within four days it escapes entirely. The tetanus poison is destroyed by a temperature of 65° C. maintained for five minutes, or 60° for twenty minutes, or 55° for an hour and a half; in the incubating oven at 37° C. it gradually loses its toxic potency; in diffuse daylight, also, its toxic power is gradually lost; in a cool, dark place it retains its original potency indefinitely; in direct sunlight it is completely destroyed in from fifteen to eighteen hours; it is not injured by being largely diluted with distilled water; it is destroyed in an hour by hydrochloric acid in the proportion of 0.55 per cent; terchloride of iodine destroys it in the proportion of 0.5 per cent; cresol in one per cent—one hour's exposure. In general it is destroyed by acids and by alkalies. Blood serum from cattle, horses, sheep, rabbits, rats, or guinea-pigs does not modify its toxic properties.

Brieger (1886) first succeeded in obtaining from impure cultures of the tetanus bacillus a crystallizable toxic substance, called by him *tetanin*, which was found to kill small animals in very minute doses and with the characteristic symptoms of tetanus. More recently Kitasato and Weyl have obtained the same substance, by following Brieger's method, from a pure culture of this bacillus. From a bouillon made from one and one-fourth kilogrammes of lean beef, with the addition of twenty-five grammes of peptone, they obtained 1.7118 grammes of hydrochlorate of tetanin. This proved fatal to white mice in six hours in the dose of 0.05 gramme, and a dose of 0.105 gramme caused characteristic tetanic convulsions and death within an hour. The bacteriologists last named also obtained from their cultures the *tetanotoxin* of Brieger. Two mice were inoculated subcutaneously with 0.003 gramme of this substance; one died at the end of five hours without the development of tetanic symptoms; the other survived. In addition to these substances, indol, phenol, and butyric acid were demonstrated to be present in cultures of the tetanus bacillus.

The more recent researches of Brieger and Fränkel, and of Kitasato, show that the toxic ptomaïn discovered by Brieger in 1886 is not the substance to which cultures of the tetanus bacillus owe their great and peculiar pathogenic power. The distinguished German chemist and his associate have succeeded in isolating from tetanus cultures a *toxalbumin* which is far more deadly than tetanin.

Brieger and Cohn in more recent investigations (1893) relating to the toxic products of the tetanus bacillus have arrived at the following results: The cultures were made in veal bouillon containing one per cent of peptone and one-fifth per cent of chloride of sodium. Large quantities of the cultures in this medium were filtered through porcelain filters. The active substance was precipitated from the filtrate by means of a saturated solution of ammonium sulphate. By adding this salt in excess the precipitate is made to rise to the surface and is skimmed off with a platinum spatula. The liquid is removed by placing this upon porous porcelain plates and the crude toxin is dried in a vacuum. It still contains 6.5 per cent of ammonium sulphate. The tetanus bouillon after filtration is said to be fatal to mice in the dose of 0.00005 cubic centimetre. A litre of this bouillon gave about one gramme of the dried precipitate, which produced characteristic tetanic symptoms and death when injected into mice in the dose of 0.0000001 gramme. Kitasato in his experiments had previously obtained a tetanus bouillon which was five times as toxic as that used by Brieger and Cohn in their experiments, and which killed mice in the dose of 0.00001 cubic centimetre. The dried precipitate obtained by Brieger and Cohn contained various impurities, including a certain amount of ammonium sulphate, but was found to kill susceptible animals in the proportion of 0.0000066 gramme per kilogramme of body weight.

It was purified without loss of toxic power by placing it in a dialyzer in running water for from twenty-four to forty-eight hours, after which it was dried *in vacuo* at 20° to 22° C. The purified toxin thus obtained had a slightly yellowish color, and was in the form of transparent scales, which were odorless, tasted like gum acacia, and were easily soluble in water. The chemical reactions of this purified toxin, according to Brieger and Cohn, show that it is not a true albuminous body. When injected beneath the skin of a mouse weighing fifteen grammes, in the dose of 0.00000005 gramme, it causes its death, and one-fifth of this amount gave rise to tetanic symptoms from which the animal recovered after a time. The lethal dose for a man weighing seventy kilogrammes is estimated by the authors named to be 0.00023 gramme (0.23 milligramme). Comparing this with the most deadly vegetable alkaloids known it is nearly six hundred times as potent as atropine and one hundred and fifty times as potent as strychnine.

Fermi and Pernossi (1894), as a result of an elaborate research, have determined many of the chemical characters of the tetanus toxin. When in solution it is destroyed by a comparatively low temperature (55° C. for one hour) and by exposure to direct sunlight, but the dry powder resists a temperature of 120° C. It has not the properties of an alkaloid, as it is not dissolved by any of the usual solvents of these bodies—the only solvent thus far discovered is said to be water. It resembles the albumins and peptones in its failure to pass through a dialyzing membrane. The authors last referred to conclude their summary of results as follows:

> "The appended table shows that the tetanus poison, like that of diphtheria, in its behavior as regards the action of light, heat, chemical agents, and dialysis, as also its solvents, the agents which precipitate it, and its action upon living animals, closely resembles the poisons of serpents (Naja tripudians, Crotalus, etc.). As to the chemical nature of this group of substances, we can at present only say that they rather have the characters of collodial substances than otherwise, and more nearly resemble the albuminoid bodies than the bases. We do not, however, reject the very probable hypothesis that these toxins are acids or bases, or other very unstable, peculiar substances, which are closely united with colloidal substances, as is the case, for example, with the alkali and acid albumins and so many other albuminous bodies."

While the exact nature of the toxic substance contained in tetanus cultures has not been determined, we probably cannot, at present, do better than to continue to speak of it as a "toxalbumin."

Kitasato (1891) was not able to produce immunity in mice by inoculations with minute doses of the poison, or with a filtrate which had been exposed to various degrees of temperature by which its activity was diminished or destroyed. But immunity lasting for about two months was produced in rabbits by inoculating them with the filtrate from a culture of the tetanus bacillus, and subsequently, in the same locality, with three cubic centimetres of a one-per-cent solution of terchloride of iodine; this last solution was injected subcutaneously in the same dose at intervals of twenty-four hours for five days. Of fifteen rabbits treated in this way six proved to be immune against large doses of a virulent culture of the tetanus bacillus. The same treatment was not successful in producing immunity in mice or guinea-pigs, but the important discovery was made that a small quantity of blood (0.2 cubic centimetre) from an immune rabbit, when injected into the abdominal cavity of a mouse, gave it immunity from the effects of inoculations with the tetanus bacillus. Moreover, mice which were first inoculated with a virulent culture of the bacillus, and, after tetanic symptoms had appeared, received in the cavity of the abdomen an injection of blood serum from an immune mouse, were preserved from death. The power of the blood of an immune animal to neutralize the tetanus poison was further shown by mixing the filtrate from a virulent culture with blood serum from an immune animal and allowing it to stand for twenty-four hours; a dose three hundred times greater than would have sufficed to kill a mouse proved to be without effect after such admixtures with blood serum; as before stated, the blood serum of animals which are not immune has no effect upon the poison. The duration of immunity induced in this way was from forty to fifty days. Blood serum from an immune rabbit, preserved in a cool, dark room, retains its power of neutralizing the tetanus poison for about a week, after which time it gradually loses it. Having found that chickens have a natural immunity against tetanus, Kitasato made experiments to ascertain whether their blood serum would also neutralize the tetanus poison; the result was negative.

That the tetanus poison is present in the blood of individuals who die from tetanus has been proved by Kitasato by injecting a small quantity (0.2 to 0.3 cubic centimetre) of blood from the heart of a fresh cadaver into mice; the animals develop typical tetanic symptoms and die in from twenty hours to three days.

Tizzoni and Cattani have (1891) reported results similar to those obtain by Kitasato. By repeated inoculations with gradually increasing doses of the tetanus poison they succeeded in making a dog and two pigeons immune, and found that blood serum from this immune dog, in very small amount, completely destroyed the toxic power of a filtrate from cultures of the tetanus bacillus—one to two drops of serum neutralized 0.5 cubic centimetre of filtrate after fifteen to twenty minutes' contact. They also ascertained that small amounts of blood serum from this immune dog injected into other dogs or white mice produced immunity in these animals; but they were not able to produce immunity in guinea-pigs or rabbits by the same method.

In a later communication (May, 1891) Tizzoni and Cattani give an account of their experiments made with a view to determining the nature of the substance in the blood serum of an immune animal which has the power of destroying the toxalbumin of tetanus—"tetanus antitoxin." They found, in the first place, that this antitoxin in blood serum is destroyed in half an hour by a temperature of 68° C.; further, that it does not pass through a dialyzing membrane; that it is destroyed by acids and alkalies. As a result of their researches they conclude that it is an albuminous substance having the nature of an enzyme.

Vaillard has succeeded in producing immunity in rabbits by repeated injections into the circulation of filtered cultures—in all twenty cubic centimetres—which had been exposed for one hour to a temperature of 60° C. At a temperature of 65° C. both the toxic and the immunizing action is destroyed.

Behring (1892) gives the following account of a method which he has successfully employed for producing immunity in large animals—especially in horses: A culture of the tetanus bacillus is made, in bouillon, of such toxic potency that 0.75 cubic centimetre will kill a rabbit in three or four days. To two hundred cubic centimetres of this culture he adds carbolic acid in the proportion of 0.5 per cent for the purpose of preserving it. The horse first receives a subcutaneous injection of ten cubic centimetres of this culture fluid to which terchloride of iodine (ICl_3) has been added in the proportion of 0.25 per cent; at the end of eight days twenty cubic centimetres of the same mixture are given; again in eight days the dose is repeated; then, after an interval of three days, thirty cubic centimetres of the same mixture. Following this, at an interval of eight days, he gives two injections of thirty cubic centimetres each of a mixture containing one-half the quantity of ICl_3 (0.175 per cent). The proportion of the iodine terchloride is then reduced to 0.125 per cent, and two doses of twenty cubic centimetres each are given. Finally the culture fluid is administered in the dose of 0.5 cubic centimetre, and this dose is doubled every five days. Before giving the first dose of culture fluid without the addition of ICl_3, the immunizing value of the blood serum of the horse is tested on mice, and if it falls below 1:100 a dose of 0.25 cubic centimetre is given instead of the larger dose (0.5 cubic centimetre) above mentioned.

Schütz (1892) has applied Behring's method to a considerable number of horses and sheep, and arrives at the conclusion that it is a reliable method of protecting these animals against infection with living tetanus bacilli and against the toxic action of filtered cultures; that the degree of immunity and the antitoxic power of the blood serum increase as larger doses are gradually given. According to Behring the immunizing value of blood serum from a horse treated in this way is very high. As tested on mice it may be 1:200,000, or even more. According to his calculations a serum having a value of 1:100,000, as tested on mice, should be given to a man weighing fifty kilogrammes in the quantity of fifty cubic centimetres, given in the course of two days, in order to insure immunity.

The same author in a subsequent paper (1892) gives details as to the method of estimating the therapeutic value of serum from an immune animal. He first calls attention to the fact that the only reagent by which the antitoxic potency of this serum can be tested is the body of a living animal. The test animal selected is the white mouse. When the statement is made that a serum has the value of 1:1,000,000, he means that by an experimental test, made upon white mice, it has been ascertained that these animals are protected from fatal infection with the minimal lethal dose of a tetanus culture by the use of 0.00002 gramme of the serum for a mouse weighing twenty grammes. For the cure of tetanus in the mouse, after the first symptoms of the disease have appeared, a dose at least one thousand times as great as the immunizing dose is required, and the more advanced the progress of the case the greater the dose must be. A serum of the strength above indicated, if used for the treatment of a case of tetanus in man, should, according to Behring, be employed in doses amounting altogether to at least one hundred cubic centimetres—given inside of twenty-four hours in doses of twenty cubic centimetres each. For persons sixteen years old he would give doses of ten cubic centimetres, and for children under six, five cubic centimetres at a dose. The serum of this strength which he had prepared for testing its curative value on man was preserved by the addition of 0.5 per cent of carbolic acid.

Rotter (1892) reports a case successfully treated by Behring's serum. In all two hundred and fifty cubic centimetres was administered subcutaneously. The case was not, however, one of the most severe forms of the disease.

Brieger and Ehrlich (1892) have succeeded in immunizing goats by means of gradually increasing doses of a culture of the tetanus bacillus in thymus bouillon. The amount given at first was 0.2 cubic centimetre, and this was gradually increased to ten cubic centimetres. At the end of thirty-seven days the animal was found to be immune against virulent cultures, and the important fact was demonstrated that the immunizing substance (antitoxin) was present in its milk. A mouse which received 0.1 cubic centimetre of the milk of this goat in the peritoneal cavity proved to be immune against infection as a result of inoculation with a tetanus culture. The immunizing value of the milk from this goat was found to be 1,600. That is, a dose of 0.2 cubic centimetre, which was equal to 1:100 of the body weight of the animal, protected a mouse from sixteen times the fatal dose of a tetanus culture. After precipitation of the casein the milk still preserved its antitoxic power unimpaired, and by concentrating it *in vacuo* a fluid was obtained which proved to have an immunizing value of 5,000.

In a later communication (1893) Brieger and Cohn give the results of additional experiments with the milk of immunized goats. Animals were chosen which were two or three years old and had given birth to young a few weeks before the inoculations were commenced. It having been previously shown by Ehrlich that the precipitated tetanus toxin from cultures could be successfully used to immunize guinea-pigs, the same substance was employed in these experiments. The treatment was commenced with a dose of 0.00001 gramme, which was carefully increased to 0.00007 gramme, the injections being made at intervals of four days. But this proved to be too much, and the animal died of typical tetanus after the last dose. In a subsequent experiment Brieger and Cohn succeeded in immunizing a goat in a month and a half so that the animal finally withstood a dose of 0.06 gramme, but this animal ceased to give milk, became anæmic, and finally died.

The authors therefore resorted to a different method which had previously been successfully employed by Ehrlich, Behring, and others. Cultures of the tetanus bacillus in bouillon were heated to 65° C. for half an hour, and then used for immunizing two goats. After five weeks' treatment the animals resisted doses of the precipitated toxin, which were gradually increased to ten grammes, at which time the treatment had been carried on for nearly six months and the antitoxic value of the milk was found to be 90,000 immunization units.

The method of determining antitoxic values adopted by Brieger and Cohn is the following: They had found by carefully conducted experiments that their precipitated toxin (*Rohgifte*) killed a mouse weighing twenty grammes in the dose of 0.0000003 gramme, but failed to kill when injected in the dose of 0.0000002 gramme. The first-mentioned dose was therefore accepted as the minimum fatal dose for an animal weighing eighteen to twenty grammes, and the object in view was to find the minimum amount of milk required to prevent the toxic action of such a dose.

The antitoxin was obtained from the goat's milk by precipitation with ammonium sulphate, thirty-two per cent; the precipitate was again dissolved and treated with a solution of basic acetate of lead; this salt does not precipitate the antitoxin when the solution is slightly alkaline; the voluminous precipitate produced by the lead acetate is filtered out and repeatedly washed with water; the filtered fluid and wash water are again treated with ammonium sulphate, added to saturation, and the resulting precipitate is dissolved in a small quantity of water; a precipitate is again obtained by saturation with ammonium sulphate, and this is dried upon porcelain plates in a vacuum. The ammonium sulphate remaining could not be removed by dialysis, as experiment showed that a considerable loss of the antitoxin occurred in a dialyzer placed in running water. But by shaking up the dry powder in chloroform the heavy salt sank to the bottom and the purified antitoxin floated on the surface and could be recovered by skimming it off. The powder thus obtained consisted of a mixture of various substances, including the antitoxin, and when obtained from milk having an antitoxic value of 90,000 it was found to have a value of 25,000,000 immunization units. By further purification a still higher value was obtained (55,000,000). In experiments on mice a dose ten thousand times as great as was necessary to produce immunity proved to exercise a curative power—*i.e.*, a dose of 0.02 gramme for a mouse weighing twenty grammes saved it from being killed by double the minimum fatal dose of the tetanus toxin, after tetanic symptoms had been developed.

Reference has been made to the production of immunity by the use of cultures made in thymus bouillon. This was made known through the experiments of Brieger, Kitasato, and Wassermann (1892). The thymus bouillon is made from the thymus glands of calves, which are chopped fine in a hash machine and covered with an equal volume of distilled water. The mixture is stirred for some time and then placed in an ice chest for twelve hours; the liquid is then obtained by filtration through gauze with pressure—by means of a flesh-press machine. A turbid, slimy fluid is thus obtained, which is diluted with an equal volume of water and made slightly alkaline by the addition of soda solution. It is then sterilized at 100° C. for fifteen minutes. As a result of this the liquid has a grayish-brown color, and some large flocculi in suspension, which are removed by passing it through fine linen. The fluid is then of a milky opalescence. It is next placed in test tubes and again sterilized. The tetanus bacillus when cultivated in this medium does not form spores, and the toxic potency of the culture is very much reduced—1:5,000 to 1:3,000 of the toxic potency manifested by cultures of the same bacillus in ordinary media. Inoculations with cultures in thymus bouillon were found to kill mice in the dose of 0.5 cubic centimetre, while smaller amounts failed to kill and caused the animals to be immune. A culture in ordinary bouillon was fatal to mice in the dose of 0.001 cubic centimetre.

Experiments on rabbits (thirty-five) gave a uniformly successful result in immunizing these animals. Immunity was established in the course of two weeks, and the blood serum of these animals tested on mice showed an antitoxic value of 1,000.

Reference has already been made to the earlier researches of the Italian investigators, Tizzoni and Cattani. These have been followed by additional investigations, the results of which have been reported in numerous published papers. The authors named have ascertained that when kept in a cool place (15° to 25° C.) the blood serum of immune rabbits retains its antitoxic power for several months, and the antitoxin, obtained by precipitation with alcohol, kept in a dry condition for more than ten months, was found to preserve its original activity.

Having succeeded in their earlier experiments in immunizing rabbits and dogs, Tizzoni and Cattani (in 1893) proceeded to experiment upon horses, and were equally successful with these animals. As a result of numerous injections with an attenuated virus, continued for a period of ninety-seven days, they established an immunity which was tested by inoculating the animal with ten cubic centimetres of a gelatin culture, of which one two-hundredth part of a drop killed a white mouse. The antitoxic value of the blood serum of this horse was 1:5,000,000—*i.e.*, one gramme of this serum would immunize five million grammes of mice, or two hundred and fifty thousand mice weighing twenty grammes each. In a later communication (1894) the authors named report that after freely bleeding immunized horses, and allowing them to rest for one or two months, and then again treating them with small doses of tetanus cultures, the blood serum soon becomes as active as before the bleeding. The greatest antitoxic power was manifested from twenty to twenty-three days after the completion of the protective inoculations, and a serum was obtained possessing a value of 1:10,000,000. According to the authors named the precipitated (by alcohol) and purified antitoxin from such a serum, judging from their experiments on lower animals, should cure a case of tetanus in man in the dose of from forty to fifty centigrammes.

The authors last mentioned have reported (1892) that the young of immune parents have a certain degree of inherited immunity. And the more recent experiments of Ehrlich and Hübener have confirmed this so far as the inheritance of immunity from the mother (in mice) is concerned; but their results did not show any immunity in the young when only the father had been rendered immune; and the immunity inherited from the mother only lasted for two or three months after birth.

TUBERCULOSIS.

Metchnikoff states that when kept at a temperature of 42° C. for some time the tubercle bacillus undergoes a notable diminution in its pathogenic power, and that when kept at a temperature of 43° to 44° C. it after a time only induces a local abscess when injected subcutaneously into guinea-pigs. The experiments of Löte also indicate that an "attenuation of virulence" has occurred in the cultures preserved in Koch's laboratory, originating in 1882 from the lungs of a tuberculous ape. The author named made experiments with cultures from this source (ninetieth to ninety-fifth successive cultures), and at the same time with a culture obtained from Roux, of Pasteur's laboratory. Rabbits inoculated with cultures from the last-mentioned source developed a hectic fever at the end of two weeks, and died tuberculous at the end of twenty-one to thirty-nine days. Twelve rabbits were inoculated with the cultures from Koch's laboratory; the injections were made either subcutaneously, or into a vein, or into the pleural cavity, or into the cavity of the abdomen. No elevation of temperature occurred in any of the animals, and they were found at the end of a month to have increased in weight. At the end of six weeks one of them was killed and tubercular nodules were found in various organs. The remaining animals were killed at the end of one hundred and forty-four to one hundred and forty-eight days. The two inoculated subcutaneously presented no sign of general tuberculosis, but a small yellow nodule containing bacilli was found at the point of inoculation. Those inoculated by injection into a vein showed one or two nodules in the lungs containing a few bacilli. In Koch's original experiments rabbits were killed by intravenous inoculation of his cultures in from thirteen to thirty-one days. That this attenuation of virulence depends upon a diminished production of toxic product to which the bacillus owes its pathogenic power appears to be very certain, in view of the fact that the late cultures in a series have a more vigorous and abundant development than the more pathogenic cultures obtained directly from the animal body.

The discovery by Koch of a *toxin* in cultures of this bacillus, which is soluble in glycerin, and which in very minute doses produces febrile reaction and other decided symptoms when injected subcutaneously into tuberculous animals, must rank as one of the first importance in scientific medicine, whatever the final verdict may be as to its therapeutic value in tuberculous diseases in man.

The toxic substance contained in Koch's glycerin extract from cultures of the tubercle bacillus, now generally known under the name of tuberculin, is soluble in water, insoluble in alcohol, and passes readily through dialyzing membranes. It is not destroyed by the boiling temperature. According to the chemical examination of Jolles, the "lymph" contains fifty per cent of water and does not contain alkaloids or cyanogen compounds. It contains albuminates, which are thrown down as a voluminous white precipitate by tannic acid, and are redissolved by hot water containing sodium chloride and very dilute potash solution. The elementary analysis gave N 5.90 per cent, C 35.19 per cent, and H 7.02 per cent. The results obtained are believed to show that the active substance present in the lymph is a toxalbumin. In experiments made with Koch's lymph in Pasteur's laboratory by Bardach, a very decided elevation of temperature was produced in tuberculous guinea-pigs by the subcutaneous injection of 0.1 gramme, and a fatal result by the injection of 0.2 to 0.5 gramme. In man a decided febrile reaction is produced in tuberculous patients by very much smaller doses—0.001 cubic centimetre.

Hammerschlag, in his chemical researches, found that the tubercle bacillus yields a larger proportion of substances soluble in alcohol and ether than any other bacilli tested (twenty-seven per cent). The alcoholic extract contains fat, lecithin, and a toxic substance which produces convulsions in rabbits and guinea-pigs. The portion insoluble in alcohol and ether contains cellulose and an albuminoid substance. No ptomains were found, but a toxalbumin was isolated, which caused an elevation of temperature in rabbits of 1° to 2° C., lasting for a day or two.

Koch (1891) has given a full account of his method of preparing crude tuberculin, and also the process by which he obtains from this a tuberculin which appears to be pure, or nearly so. To obtain considerable quantities of the crude product the tubercle bacillus is cultivated in an infusion of calves' flesh, or of beef extract to which one per cent of peptone and four or five per cent of glycerin have been added. This culture liquid must be made slightly alkaline, and it is placed in flasks with a flat bottom, which should not be more than half filled—thirty to fifty cubic centimetres. The inoculation is made upon the surface with small masses from a culture upon blood serum or glycerin agar. By accident Koch discovered that these masses floating upon the surface give rise to an abundant development, and to the formation of a tolerably thick and dry white layer, which finally covers the entire surface. At the end of six to eight weeks development ceases, and the layer after a time sinks to the bottom, breaking up meanwhile into fragments. These cultures, after their purity has been tested by a microscopical examination, are poured into a suitable vessel and evaporated to one-tenth the original volume over a water bath. The liquid is then filtered through procelain. The crude tuberculin obtained by this process contains from forty to fifty per cent of glycerin, and consequently is not a suitable medium for the development of saprophytic bacteria, if they should by accident be introduced into it. It keeps well and preserves its activity indefinitely.

From this crude tuberculin Koch has obtained a white precipitate with sixty-per-cent alcohol which has the active properties of the crude tuberculin as originally prepared. This is fatal to tuberculous guinea-pigs in doses of two to ten milligrammes. It is soluble in water and in glycerin, and has the chemical reactions of an albuminous body. In preparing it one volume and a half of absolute alcohol is added to one volume of the crude tuberculin, and, after stirring it to secure uniform admixture, this is put aside for twenty-four hours. At the end of this time a flocculent deposit will be seen at the bottom of the vessel. The fluid above this is carefully poured off; and an equal quantity of sixty-per-cent alcohol is poured into the vessel for the purpose of washing the precipitate. This is again allowed to settle, and the procedure is repeated three or four times, after which the precipitate is washed with absolute alcohol. It is then placed upon a filter and dried in a vacuum exsiccator.

The "tuberculocidin" of Klebs is a purified tuberculin obtained by precipitation with alcohol. The precipitate is washed in chloroform and then dissolved in a mixture of carbolic acid and glycerin.

Bujwid (1894) prepares tuberculin as follows: He uses cultures on glycerin agar or in glycerin bouillon which have been kept at a suitable temperature for five to eight weeks. The glycerin-agar cultures are treated with distilled water by which the tuberculin is extracted. After adding the water the test tubes are kept in a cool place for twenty-four hours, and this is repeated two or three times. The extract from the agar cultures or the bouillon cultures is then sterilized by exposure for from five to ten minutes to a temperature of 100° C.; then filtered through a Chamberland filter; then evaporated at a low temperature to a syrup-like consistence. When this crude tuberculin is dropped into ten times its volume of strong alcohol a brown precipitate is thrown down which contains the active principle. From the tubercle bacilli obtained by filtering his cultures Bujwid also obtained an active substance which in doses of two milligrammes caused an elevation of 2° C. in the temperature of an infected guinea-pig. This substance was obtained by digesting the bacilli for two months in glycerin and water (three per cent of glycerin), filtering and evaporating the extract, and precipitation in six volumes of ninety-five-per-cent alcohol. The precipitate when dried was in the form of a white powder.

Helman (1894) obtains tuberculin from potato cultures. The sections of potato are neutralized by leaving them for half an hour in a solution of one-half to one per cent of bicarbonate of soda, after which they are sterilized for twenty minutes in the autoclave at 120° C. The best results were obtained when the potatoes were wet with a five-per-cent solution of glycerin. The sections of potato were placed in Petri's dishes upon blotting paper wet with a sublimate solution, and the dishes containing the cultures were surrounded with cotton wet with the same solution. The cultures were subsequently treated with distilled water, to extract the active principle, which was also obtained from the bacilli by mixing them with glycerin in the proportion of 1:10.

Numerous experiments have been made with dead tubercle bacilli, as well as with the toxic products developed in cultures. Héricourt and Richet (1890) found by experiment that old cultures heated to 80° C., several days in succession, when injected into a vein in rabbits, in the dose of ten to twenty cubic centimetres, caused the death of these animals. Smaller doses from which the animals recovered seemed to make them less susceptible to infection than control animals, but the number of experiments was too limited to establish this as a fact. In a subsequent (1891) communication the authors named claim to have succeeded in immunizing rabbits by injecting filtered and sterilized cultures of the tubercle bacillus, either subcutaneously (five to fifteen cubic centimetres) or into a vein (twenty to forty drops). The injections were repeated every second or third day for a period of fifteen days, after which the test inoculation was made with a culture, obtained from a tuberculous cow in one series, and from tuberculous fowls in another. Four vaccinated rabbits in the first series escaped general tuberculosis, while four out of eight control animals died tuberculous. In the second series five vaccinated animals resisted infection and three out of four control animals died tuberculous.

De Schweinitz (1894) has reported the results of experiments with attenuated cultures of the tubercle bacillus, and has, apparently, succeeded in conferring immunity upon guinea-pigs by inoculations with such cultures.

Klebs (1891), in experiments on guinea-pigs and rabbits, convinced himself that the fatal result of an inoculation with tubercle bacilli (in the cavity of the abdomen or subcutaneously in guinea-pigs, and in the eye in rabbits) was greatly delayed by injections of Koch's tuberculin (0.3 to 0.5 cubic centimetre) either before or after infection.

Baumgarten (1891), in experiments upon rabbits inoculated with tubercle bacilli in the anterior chamber of the eye, failed to obtain favorable results from treatment with Koch's tuberculin given in considerable doses (0.5 to one gramme) either before or after infection.

The results reported in the same year by Gramatschikoff, by Popoff, by Alexander, and by Gasparini and Mercanti, were also unfavorable as regards an immunizing or curative effect from inoculations of tuberculin in rabbits. Dönitz, on the contrary, arrives at the conclusion that when early treatment is instituted iris tuberculosis may be arrested and cured, and the more recent experiments of Trudeau (1893) give support to this conclusion. Baumgarten, however, insists that the tuberculin treatment does not prevent metastasis to the lungs after inoculations in the anterior chamber of the eye.

Pfuhl (1891) treated forty-seven infected guinea-pigs, and at the date of his report forty-four had died tuberculous, but the date of death was somewhat postponed by the treatment. The animals not treated succumbed at the end of eight weeks (average of all controls), and those treated with small doses of tuberculin lived, on the average, ten weeks. With larger doses still more favorable results were obtained—four lived on an average twelve weeks, and three were still living, eleven, fifteen, and sixteen weeks after infection, at the date of publication.

Kitasato (1892) also obtained favorable results in the treatment of infected guinea-pigs, and arrives at the conclusion that guinea-pigs which have been cured by the treatment are not susceptible to a second infection, for a certain time at least.

Bujwid (1892), in experiments upon guinea-pigs, found that infected animals which received from 0.05 to 0.1 gramme of tuberculin within three hours showed an elevation of temperature of 1.5° to 2° C. Thirteen infected guinea-pigs treated with tuberculin lived from two and a half to eight months, while all of the control animals (eighteen) died in from six to nine weeks. The animal which survived eight months was found not to be tuberculous, but presented evidence of recovery from a former tuberculous process. In two rabbits inoculated in the anterior chamber the iris tuberculosis was favorably influenced by the tuberculin treatment, but general infection occurred, and the animals died about the same time as the controls. Three apes were treated without any apparent result; they all died within two months after infection.

The experiments of Gramatschikoff, Czaplewski, and Roloff, and of Yamagiva, published in 1892, show that the tuberculin treatment does not cure tuberculous infection in inoculated guinea-pigs and rabbits, and that the bacilli retain their vitality in such animals in spite of the most persistent treatment.

Héricourt and Richet (1892), in experiments made for the purpose of immunizing animals against tuberculous infection, failed to obtain positive results in the most susceptible species—guinea-pigs, rabbits, and apes—but claim to have succeeded in immunizing dogs by intravenous injections of cultures of the bacillus of tuberculosis in fowls. Animals which had been so treated after an interval of two to six months received an intravenous injection of one cubic centimetre of a culture of the bacillus tuberculosis from man. This was fatal to "non-vaccinated" dogs, as a rule, in about three weeks, but the "vaccinated" animals survived the injection.

The results obtained by Trudeau (1893) are of such interest that we shall quote *in extenso* what he says with reference to preventive inoculations:

> "Antitubercular inoculation was first tried by Falk in 1883, and all attempts in this direction have resulted until recently in but an unbroken record of failures. In 1890 I added my name to the list of those who found it impossible to produce immunity in animals by this method. In 1890, Martin and Grancher, and Courmont and Dor, claimed to have produced in rabbits a certain degree of immunity by previous inoculation, after Pasteur's hydrophobia method, of avian tubercle bacilli of graded and increasing virulence. These vaccinations were, however, frequently fatal to the animals, and the immunity obtained was but slight. Richet and Héricourt have since claimed to produce complete immunity in dogs by intravenous inoculations of bird tubercle bacilli. These experimenters found that though harmless to the dog when first derived from the chicken, bird bacilli, by long cultivation in liquid media, become pathogenic for this animal, and by thus grading the virulence of the injections complete immunity against any form of tubercular infection was produced in the dog. As yet these striking results have not been confirmed. The animals which I now present to you illustrate an attempt I have made along the same line to produce immunity in the rabbit. Cultures grown directly from the chicken's lesions in bouillon for, first, five weeks, then six months, were twice injected subcutaneously at intervals of twenty-one days in doses of 0.025 and 0.05, and a third injection of a still older culture was occasionally given. About one in four of the rabbits died within three months, profoundly emaciated, but without any visible tubercular lesions. The remaining animals recovered aud were apparently in good health, when, together with an equal number of controls, they were inoculated in the anterior chamber of the eye with cultures of Koch's bacillus derived from the tuberculous lesions of the rabbit, and cultivated about three months on glycerin-agar. The results of these inoculations present many points of interest. In the controls, as is usually the case, if the operation has been done carefully and aseptically, and with a moderate amount of dilute virus, two days after the introduction of the virulent material in the eye little or no irritation is observed, and little is to be noticed for two weeks, when a steadily increasing vascularity manifests itself, small tubercles appear on the iris, which gradually coalesce and become cheesy, intense iritis and general inflammation of the structures of the eye develop, the inoculation wound becomes cheesy, and in six to eight weeks the eye is more or less completely destroyed and the inflammation begins to subside. The disease, however, remains generally localized in the eye for many months, and even permanently. In the vaccinated animals, on the contrary, the introduction of the virulent bacilli at once gives rise to a marked degree of irritation. On the second day the vessels of the conjunctiva are tortuous and enlarged, whitish specks of fibrinous-looking exudation appear in the iris and in the anterior chamber, and more or less intense iritis supervenes; but at the end of the second to the third week, when the eyes of the controls begin to show progressive and steadily increasing evidence of inflammatory reaction, the irritation in those of the vaccinated animals begins slowly to subside and the eyes to mend. The vascularity is less, the whitish spots of fibrinous material

appear smaller, the structures of the eye become clearer, the inoculation wound is but a bluish fibrous scar, until in from six to twelve weeks, in successful cases, all irritation has disappeared and the eyes present, as in the animals I now show you, but fibrous evidence of the traumatism and the inflammatory processes which have been set up by the inoculation. In all the controls, as you see, the inoculation wound is cheesy and the cornea and iris are more or less destroyed by tubercle and cheesy areas.

"Some of the protected animals slowly relapse, and the one I now show you has small tubercles growing on the iris; but even in such eyes the entire absence of caseation is noticeable, and the disease progresses almost imperceptibly. I have repeated this experiment on three sets of rabbits with about the same results each time. The vaccinations as practised are of themselves, in some instances, fatal; but the fact remains that where recovery takes place a marked degree of immunity has been acquired. I do not lay any claim, therefore, to have produced a complete or permanent immunity by a safe method, but it seems to me that these eyes constitute a scientific demonstration of the fact that in rabbits preventive inoculation of bird-tubercle bacilli can retard, and even abort, an otherwise progressive localized tubercular process so completely as to prevent destruction of the tissues threatened, and that the future study of anti-tubercular inoculation may not be as entirely hopeless as it has until recently appeared."

TYPHOID FEVER.

Brieger (1885) found in cultures of the typhoid bacillus small amounts of volatile fat acids, and when grape sugar has been added to the culture medium lactic acid. He also obtained a highly alkaline basic substance possessing toxic properties which he named typhotoxin ($C_7H_{17}NO_2$). This he supposes to be the specific product to which the pathogenic action of the bacillus is due. It produces in mice and guinea-pigs salivation, paralysis, dilated pupils, diarrhœa, and death.

More recent experiments by Pfeiffer (1894) lead him to conclude that the specific poison of the typhoid bacillus is not present in filtered cultures, but is closely associated with the bacterial cells. According to Pfeiffer the bacillus may be killed by a temperature of 54° C. without injury to this toxic substance. The fatal dose of the dead bacilli is from three to four milligrammes per one hundred grammes of body weight for guinea-pigs. Susceptible animals may be immunized by means of this toxic substance, and their blood is found to contain an antitoxin which has a specific bactericidal action upon the typhoid bacillus. But, according to Pfeiffer, the blood serum of animals immunized in this way does not differ from normal serum in its action on bacillus coli communis and other species of bacteria. These results are believed, by the author referred to, to settle the question of the specific character of the typhoid bacillus, and to differentiate it from nearly allied species. The presence of a typhoid antitoxin in the blood serum of individuals who have recently suffered an attack of typhoid fever has also been demonstrated by Pfeiffer.

Chantemesse and Widal (1888) first showed by experiment that susceptible animals could be made immune against the pathogenic action of this bacillus by the subcutaneous injection of sterilized cultures. Having found that four drops of a bouillon culture, three days old, injected into the peritoneal cavity of white mice caused the death of these animals within thirty-six hours, they proceeded to inject small quantities (one-half cubic centimetre) of a culture which had been sterilized by heat, and found that after several such protective inoculations the mice no longer succumbed to infection by an unsterilized culture.

In experiments made upon rabbits, Bitter (1892) arrived at the conclusion that the immunity which he produced in these animals by the intravenous injection of concentrated sterilized (by filtration) cultures was due to the presence of an antitoxin in the blood of the immune animals. Having found that control animals were killed by intravenous injections of one cubic centimetre of his concentrated solution of the products of the typhoid bacillus, he added to twice this amount of the toxic solution a certain quantity (?) of blood serum from an immune rabbit, and injected the mixture into the circulation of rabbits with a negative result. Control experiments in which the toxic solution was mixed with blood serum from non-immune animals showed that this had no antitoxic effect, and the animals died. Bruschettini obtained (1892) similar results in his experiments upon rabbits with cultures sterilized by heat (60° C.). He concludes from his experiments that the blood serum of rabbits immunized in this way not only possesses antitoxic properties, but that it has greater germicidal potency for the typhoid bacillus than the blood of normal rabbits.

Stern (1892) has made experiments to determine whether the blood of recent convalescents from typhoid has greater germicidal power for the typhoid bacillus than that of other individuals. The result showed that the blood serum from persons who had recently recovered from typhoid fever had no increased germicidal power, but rather showed diminished potency for the destruction of typhoid bacilli. But blood from a man who had suffered an attack seventeen and a half years previously was found to have unusual bactericidal power, although it did not protect white mice from typhoid infection. On the other hand, blood from recent convalescents served to immunize white mice, thus indicating the presence of an antitoxin. This is also shown by the experiments of Chantemesse and Widal (1892), who report their success in immunizing susceptible animals by injecting the blood serum of other animals previously made immune by repeated injections of sterilized (by heat) cultures. The authors last named have also tested the blood serum of typhoid-fever patients, of recent convalescents from the disease, and of persons who had suffered an attack some years before the experiment was made. The experiments were made upon guinea-pigs. The authors conclude that "in general the guinea-pig is immunized against the action of virulent typhoid cultures by the subcutaneous injection of a small quantity of serum of persons who have suffered an attack of the disease, no matter how remote." But this immunity was shown to be of short duration, and quite different from that induced by the injection of sterilized cultures, which does not immediately follow the introduction of the toxic substances, but requires a certain number of days for its development. The degree of immunity is said by the authors last named to depend to a considerable extent upon the dose given, and the animals treated in this way still resisted virulent cultures at the end of two months. On the other hand, injections of blood serum from immune individuals were effective in doses of a single cubic centimetre, within a few hours, and the immunity conferred had a comparatively brief duration.

Protective inoculations in man have been practised on quite a large scale by surgeons of the English army in India and in South Africa. The method of Wright has been followed in preparing sterile cultures for inoculation. Cultures in bouillon are made and kept in the incubator at 37° C. for two or three weeks. The cultures are then drawn into small glass tubes, which are sealed by heat. The tubes are placed in a vessel containing cold water, and the temperature is gradually raised to 60° C., where it is maintained for five minutes. Plantings in a culture medium are made from these tubes to make sure that sterilization is complete. As a further protection against the introduction of living bacteria, one-half per cent of lysol may be added to the sterilized culture. The amount used for protective inoculations in man has been fixed at two-fifths of the minimum amount, which would be fatal to a guinea-pig weighing two hundred and fifty grammes. The inoculation gives rise to a well-marked local reaction, which does not result in suppuration, and to more or less pronounced general disturbance. Usually this is slight, but sometimes rigors, nausea, and a tendency to syncope occur. That these inoculations are not without effect is shown by the fact that the blood serum of an inoculated individual exercises a marked agglutinating action upon the typhoid bacillus in a recent culture (Widal reaction). This is said to be equal to that resulting from an attack of typhoid fever. Cameron, after an inoculation practised upon himself, found that at the end of twenty days his blood serum exhibited an agglutinating power forty times greater than that of normal blood. In practice it has been found advisable to repeat the inoculation at the end of a week. Wright reports that among 11,295 British soldiers inoculated in India, the percentage of those who subsequently contracted typhoid fever was 0.95, while 2.5 per cent of those not inoculated suffered an attack of this disease. According to Foulerton the soldiers in South Africa, during the Boer war, who have been inoculated have contracted typhoid fever in the proportion of six per thousand, while those not inoculated have suffered to the extent of nine per thousand. How much value should be attached to these statistics it is difficult to say, on account of the numerous factors which are likely to influence the result. Thus a command on the march in a sparsely inhabited country would be much less liable to suffer from typhoid fever than another located in a town and remaining for a considerable time on the same camping ground. In a recent report (February, 1901) Professor Wright states that of 539 officers, men, and women connected with the Fifteenth Hussars at Meerut, India, 360 received protective inoculation in England against typhoid fever and 179 did not. Of the former 2 (0.55 per cent) were admitted to the hospital, suffering from typhoid fever, with 1 death (0.27 per cent); while of the latter 11 (6.14 per cent were attacked by the fever, with 6 deaths (3.35 per cent).

It is evident that, while the results reported are encouraging, this method should not be relied upon as a substitute for those sanitary measures which must be our main reliance for the prevention of epidemics of this disease, viz., sterilization of drinking-water, disinfection of excreta, sanitary police of camps, etc.

V.

PYOGENIC BACTERIA.

THE demonstration made by Ogston, Rosenbach, Passet, and others that micrococci are constantly present in the pus of acute abscesses, led to the inference that there can be no pus formation in the absence of microörganisms of this class. But it is now well established, by the experiments of Grawitz, De Bary, Steinhaus, Scheurlen, Kaufmann, and others, that this inference was a mistaken one, and that certain chemical substances introduced beneath the skin give rise to pus formation quite independently of bacteria. Among the substances tested which have given a positive result are nitrate of silver, oil of turpentine, strong liquor ammoniæ, cadaverin, etc. The demonstration has also been made by numerous investigators that cultures of pus cocci, when sterilized by heat, still give rise to pus formation when injected subcutaneously. This was first established by Pasteur in 1878, who found that sterilized cultures of his "microbe générateur du pus" induced suppuration as well as cultures containing the living microbe. This fact has since been confirmed, as regards the pus staphylococci and various bacilli, by a number of bacteriologists. Wyssokowitsch produced abscesses containing sterile pus by injecting subcutaneously agar cultures of the anthrax bacillus sterilized by heat. Buchner obtained similar results in a series of forty experiments from the injection of sterilized cultures of Friedländer's bacillus ("pneumococcus"), and has shown that the pus-forming property belongs to the bacterial cells and not to a soluble chemical substance produced by them. When cultures were filtered by means of a Chamberlain filter the clear fluid which passed through the porous porcelain was without effect, while the dead bacteria retained by the filter produced aseptic pus infiltration in the subcutaneous tissues within forty-eight hours after having been injected. Subsequent experiments gave similar results with seventeen different species tested, including Staphylococcus pyogenes aureus, Staphylococcus cereus flavus, Sarcina aurantiaca, Bacillus prodigiosus, Bacillus Fitzianus, Bacillus subtilis, Bacillus coli communis, Bacillus acidi lactici, etc. From the experiments made to determine the exact cause of pus formation following the injection of sterilized cultures Buchner arrives at the conclusion that it is due to the albuminous contents of the bacterial cells.

While it is demonstrated that a large number of microörganisms, either living or in sterilized cultures, may give rise to the formation of pus, the extended researches of Rosenbach, Passet, and other bacteriologists show that few species are usually concerned in the formation of acute abscesses, furuncles, etc., in man. Of these the two most important, by reason of their frequent occurrence and pathogenic power, are Staphylococcus pyogenes aureus and Streptococcus pyogenes; next to these comes Staphylococcus pyogenes albus, and the following species are occasionally found: Staphylococcus pyogenes citreus, Staphylococcus cereus flavus, Staphylococcus cereus albus, Micrococcus tenuis, Bacillus pyogenes fœtidus, Micrococcus tetragenus, Micrococcus pneumoniæ crouposæ. Two or more species are often found in the same abscess; thus Passet, in thirty-three cases of acute abscess, found Staphylococcus aureus and albus associated in eleven, albus alone in four, albus and citreus in two, Streptococcus pyogenes alone in eight, albus and streptococcus in one, and albus, citreus, and streptococcus in one. Hoffa found, in twenty-two cases of inguinal bubo, aureus in ten, albus in nine, and citreus in three. Bumm, in ten cases of puerperal mastitis, found aureus in seven and Streptococcus pyogenes in three. Rosenbach found staphylococci alone sixteen times, Streptococcus pyogenes alone fifteen times, staphylococci and streptococci associated five times, and Micrococcus tenuis three times in thirty-nine acute abscesses and phlegmons examined by him.

Robb and Ghrisky have shown that under the most rigid antiseptic treatment microörganisms are constantly found attached to sutures when these are removed from wounds made by the surgeon, and that a skin abscess frequently results from the presence of the most common of these microörganisms—Staphylococcus epidermidis albus.

The authors named state their conclusions as follows:

> "A wound, at some time of its existence, always contains organisms. They occur either on the stitches or in the secretions.
>
> "The number of bacteria is influenced by the constricting action of the ligatures or drainage tube, or anything interfering with the circulation of the tissues.
>
> "The virulence of the organisms present will influence the progress of the wound.
>
> "The body temperature is invariably elevated if the bacteria are virulent; and, indeed, in cases where many of the less virulent organisms are found, almost without exception there is some rise of temperature."

The organism most frequently found—Staphylococcus epidermidis albus—has but slight virulence. Out of forty-five cases in which a bacteriological examination was made this micrococcus was obtained in pure cultures in thirty-three ; in five cases it was associated with Staphylococcus pyogenes aureus, in one case with Streptococcus pyogenes, in three cases Streptococcus pyogenes was obtained alone.

In abscesses resulting from inflammation of the middle ear the micrococcus commonly known under the name of "diplococcus pneumoniæ"—Micrococcus pneumoniæ crouposæ—has been obtained in pure cultures in a considerable number of cases when the pus has been examined immediately after paracentesis of the tympanic membrane. We shall not, however, describe this among the pyogenic bacteria, but will give an account of it in the following section (Bacteria in Croupous Pneumonia, etc.). Bacillus pyocyaneus, which is described by some authors among the pyogenic bacteria, is found only in the pus of open wounds, where its presence is evidently accidental. We shall describe it among the chromogenic saprophytes.

STAPHYLOCOCCUS PYOGENES AUREUS.

Synonym.—Micrococcus of infectious osteomyelitis (Becker).

Observed by Ogston (1881) in the pus of acute abscesses, but not differentiated from the associated staphylococci and the streptococcus of pus. Obtained by Becker from the pus of osteomyelitis (1883). Isolated from the pus of acute abscesses and accurately described by Rosenbach (1884) and by Passet (1885).

The Staphylococcus pyogenes aureus is a facultative parasite, and is the most common pyogenic micrococcus found in suppurative processes generally. But it is also a common and widely distributed saprophyte, which finds the conditions necessary for its existence on the external surface of the human body and of moist mucous membranes. This is shown by the researches of numerous bacteriologists. Thus Ullmann found it upon the skin and in the secretions of the mouth of healthy persons, and also in the dust of occupied apartments, in water, etc.; Bockhart obtained it in cultures from the surface of the body and from the dirt beneath the finger nails of healthy persons ; Biondi, Vignal, and others in the salivary secretions ; B. Fränkel in mucus from the pharynx ; Von Besser and Wright in nasal mucus ; Escherich in the alvine discharges of healthy infants ; C. Fränkel in the air ; and Lübbert in the soil. Its presence in the air, in water, or in the soil is, however, quite exceptional, and is probably to be considered the result of accident, its normal habitat as a saprophyte appearing to be rather upon the surface of the body and of mucous membranes.

Morphology.—Spherical cells having a diameter of 0.7 μ (Hadelich) to 0.9 μ (0.87 μ Passet), solitary, in pairs, or in irregular groups, occasionally in chains of three or four elements or in groups of four. The dimensions vary somewhat in different culture media, being larger in a favorable than in an unfavorable medium. The individual cells, as pointed out by Hadelich, consist of two hemispherical portions separated from each other by a very narrow cleft, which is not visible when the cells are deeply stained, but may be demonstrated, with a high power, by staining for a short time (two minutes or less) in a solution of fuchsin in aniline water.

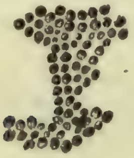

FIG. 79.—Staphylococcus pyogenes aureus, from a drawing by Rosenbach.

This micrococcus *stains* quickly in aqueous solutions of the basic aniline colors, and may also be stained with acid carmine and hæmatoxylin. It is not decolorized by iodine solution when stained with methyl violet—Gram's method.

Biological Characters.—Staphylococcus pyogenes aureus grows either in the presence or absence of oxygen, and is consequently a *facultative anaërobic.* It multiplies rapidly at a temperature of 18° to 20° C. in milk, flesh infusions, and various other liquid media, and in nutrient gelatin or agar. It *liquefies gelatin*, and in stab cultures liquefaction occurs all along the line of puncture, forming a pouch which is largest above and at the end of three or four days has extended to the full capacity of the test tube at the surface. The liquefied gelatin in this pouch is at first opaque from the presence of little agglomerations of micrococci in suspension, but after a time these are deposited and the gelatin becomes transparent. During the period of active growth the cocci accumulate near the surface of the gelatin, and, in contact with the air, the characteristic *golden-yellow pigment* is produced. By the subsidence of the colored masses of cocci from this superficial stratum a yellow deposit is gradually formed at the bottom of the pouch of liquefied gelatin (Fig. 80). This pigment, which is the principal character distinguishing the micrococcus under consideration from certain other liquefying staphylococci, is only formed in the presence of oxygen. Upon the surface of nutrient agar development occurs in the form of a moist, shining layer, with more or less wavy outlines, having at first a pale-yellow color, which soon deepens to an orange- or golden-yellow. The colonies which develop upon agar plates are spherical and opaque, and usually acquire the golden-yellow color within a few days. Colonies on gelatin plates or in Esmarch roll tubes first appear as small white dots, which later are more or less granular in appearance and present the yellow color, especially towards the centre ; but, owing to the extensive liquefaction of the gelatin caused by them, their development can only be followed for two or three days. Upon potato, at a temperature of 35° to 37° C., a rather thick, moist layer of considerable extent forms at the end of twenty-four to forty-eight hours; this is also at first of a pale-yellow, and later of an orange-yellow color. The temperature mentioned is most favorable for the rapid development of this micrococcus, although multiplication may occur at a comparatively low temperature and is tolerably abundant at the ordinary room temperature.

Cultures of the "golden staphylococcus," and especially those upon potato, give off a peculiar odor which resembles that of sour paste. When cultivated in milk it gives rise to the formation of lactic and butyric acids and to coagulation of the casein. No poisonous ptomaines or toxalbumins have been isolated from cultures of this micrococcus, but, like other liquefying bacteria, it forms a soluble peptonizing ferment, by which gelatin may be liquefied independently of the living microörganism. While the Staphylococcus aureus gives rise to the production of acids—principally lactic acid—in media containing glucose or lactose, it has also been shown by Brieger that ammonia is one of the products of its vital activity. Unlike some other pathogenic bacteria, it is able to grow in a medium having a distinctly acid reaction. A non-poisonous basic substance has been isolated by Brieger from old cultures in meat infusion which differs from any of the ptomaines obtained by him from other sources.

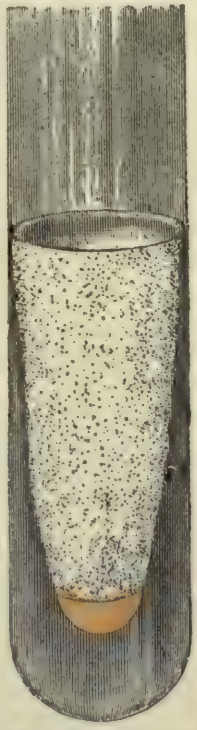

FIG. 80.—Gelatin culture of Staphylococcus pyogenes aureus (Baumgarten).

The thermal death-point of this micrococcus, in recent cultures in flesh-peptone-gelatin, as determined by the writer, is between 56° and 58° C., the time of exposure being ten minutes. When in a desiccated condition a much higher temperature is required—90° to 100° C. —for its destruction; and it retains its vitality for more than ten days when dried upon a cover glass (Passet). It retains its vitality for a long time in cultures in nutrient gelatin or agar, and may grow when transplanted from such cultures even at the end of a year.

Very numerous experiments have been made to determine the proportion of various chemical agents required to destroy the vitality or to restrain the growth of this important pyogenic micrococcus. The extended researches of Lübbert (1886) with reference to the antiseptic power of agents added to a suitable culture medium—nutrient gelatin—gave the following results: Development was prevented by the agents named in the proportion given: Nitric acid, 1 : 797; phosphoric acid, 1 : 750; boracic acid, 1 : 327; oxalic acid, 1 : 433; acetic acid, 1 : 720; citric acid, 1 : 433; lactic acid, 1 : 350; benzoic acid, 1 : 400; salicylic acid, 1 : 655; iodine dissolved with potassium iodide, 1 : 1,100; arsenite of potash, 1 : 733; mercuric chloride, 1 : 81,400; chloral hydrate, 1 : 133; carbolic acid, 1 : 814; thymol, 1 : 11,000; resorcin, 1 : 122; hydrochinon, 1 : 353; kairin, 1 : 407; antipyrin, 1 : 26; muriate of quinine, 1 : 550; muriate of morphia, 1 : 60. For the destruction of vitality very much larger amounts are required. In Bolton's experiments (1887) a one-per-cent solution of carbolic acid was successful after two hours' exposure, but two per cent failed to completely destroy vitality in the same time; one per cent of sulphate of copper was also successful, and but a single colony developed after exposure to a solution of 1 : 200. In the experiments of Gärtner and Plagge the Staphylococcus aureus in bouillon cultures is said to have been killed in a few seconds (eight) by a solution of mercuric chloride of the proportion of 1 : 1,000; Behring found it was killed by the acid sublimate solution of La Place, in the proportion of 1 : 1,000, in ten minutes; Tarnier and Vignal found that a solution of 1 : 1,000 was successful in two minutes. Abbott (1891) has shown that in the same culture there may be a considerable difference in the resisting power of the cocci, and that while frequently all are destroyed in five minutes by a 1 : 1,000 solution, it occurs quite as frequently that some may survive after an exposure of ten, twenty, and even thirty minutes.

Pathogenesis.—Subcutaneous inoculation with a small quantity of a culture of Staphylococcus pyogenes aureus is without result in rabbits, guinea-pigs, or mice, but when a considerable quantity is injected beneath the skin of a rabbit or a guinea-pig an abscess is produced, which usually results in recovery, but may give rise to general infection and the death of the animal. Injection into a vein or into the cavity of the abdomen in the animals mentioned usually induces a fatal result within a few days. The most characteristic pathological changes are found in the kidneys, which contain numerous small collections of pus and under the microscope present the appearances resulting from embolic nephritis. Many of the capillaries and some of the smaller arteries of the cortex are plugged up with thrombi consisting of micrococci. Metastatic abscesses may also be found in the joints and muscles. The micrococci may be recovered in pure cultures from the blood and the various organs; but they are not numerous in the blood, and a simple microscopical examination will often fail to demonstrate their presence.

Animals frequently survive the injection of a small quantity of a pure culture made directly into the circulation, and there is evidence that the pathogenic potency of this micrococcus may vary considerably as a result of conditions relating to its origin and cultivation in the animal body or in artificial media. When injected in considerable quantities it may be obtained in cultures from the urine, but not sooner than six or eight hours after the injection, and not until the formation of purulent foci in the kidneys has already occurred (Wyssokowitsch).

The pyogenic properties of this micrococcus have been demonstrated upon man by the experiments of Garré, of Bockhart, and of Bumm. The first-named observer inoculated a small wound at the edge of one of his finger nails with a minute quantity of a pure culture, and a subepidermal, purulent inflammation extending around the margin of the nail resulted from the inoculation. Staphylococcus aureus was recovered in cultures from the pus thus formed. A more extensive and extremely satisfactory experiment was subsequently made by Garré, who applied a considerable quantity of a pure culture obtained from the above-mentioned source—third generation—to the uninjured skin of his left forearm. At the end of four days a large carbuncle, surrounded by isolated furuncles, developed at the point where the culture had been applied. This ran the usual course, and it was several weeks before it had completely healed. No less than seventeen scars remained to give evidence of the success of the experiment.

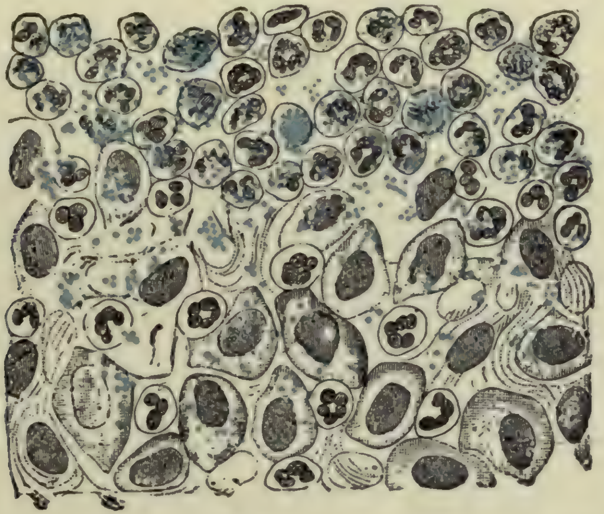

FIG. 81.—Vertical section through a subcutaneous abscess caused by inoculation with staphylococci, in the rabbit, forty-eight hours after infection; margin towards the normal tissue. × 950. (Baumgarten.)

In Bockhart's experiments a similar but milder result was obtained, the conditions having been somewhat different. A small quantity of an agar culture was suspended in 0.5-per-cent salt solution, and this was rubbed upon the uninjured skin of the left forearm. By gentle scratching with a disinfected finger nail the epithelium was removed in places over the area to which the micrococcus had been applied. As a result of this procedure numerous impetigo pustules and occasionally a genuine furuncle developed. Portions of the skin containing the smaller pustules were excised and examined microscopically. As a result of this examination Bockhart concluded that the cocci penetrate by way of the hair follicles, the sebaceous and sudoriparous glands, or, where the epidermis had been removed by scratching, directly to the deeper layers of the skin.

In Bumm's experiments, made upon himself and several other persons, Staphylococcus aureus suspended in sterilized salt solution was injected beneath the skin. An abscess resulted in every case.

The very extended researches made by bacteriologists during the past five or six years show that the golden staphylococcus is the most common pyogenic microörganism. Its presence has been demonstrated not only in furuncles and carbuncles, but also in various pustular affections of the skin and mucous membranes—impetigo, sycosis, phlyctenular conjunctivitis ; in purulent conjunctivitis and inflammation of the lacrymal sac ; in acute abscesses formed in the lymphatic glands, the parotid gland, the tonsils, the mammæ, etc. ; in metastatic abscesses and purulent collections in the joints ; in empyema ; in infectious osteomyelitis ; and in ulcerative endocarditis. The evidence relating to its presence and etiological import in the last-mentioned affections demands special consideration.

Infectious osteomyelitis appears from the researches of Becker, Rosenbach, Krause, Passet, and others, to be usually due to the presence of Staphylococcus aureus, although Kraske has shown that in certain cases this is associated with other microörganisms. Becker, who obtained this micrococcus from the pus of osteomyelitis in 1883, was the first to show by experiment that the same affection might be induced in rabbits by injecting cultures of the micrococcus into the circulation, after having crushed or fractured a bone in one of its legs. The animal usually died in from twelve to fourteen days and presented the usual appearances of osteomyelitis at the fractured point. The abundant yellowish-white pus contained the golden staphylococcus which was described by Becker, and subsequently known in the bacteriological laboratories of Germany as the "micrococcus of infectious osteomyelitis." Becker's experimental results have been confirmed by Krause and Rosenbach; and Rodet, by injecting smaller quantities of a culture into the circulation, has succeeded in producing an osteomyelitis without previous injury to the bone.

Ulcerative endocarditis has been shown by the researches of numerous bacteriologists to be occasionally accompanied by a mycotic invasion of the affected tissues by the golden staphylococcus; in other cases Streptococcus pyogenes is present. The researches of Weichselbaum, and of E. Fränkel and Sänger, also show that it is present in a certain proportion of the cases, at least, of endocarditis verrucosa, although in smaller numbers. That the diseased condition of the cardiac valves in ulcerative endocarditis is due to mycotic invasion is now generally admitted and is supported by experimental evidence. Rosenbach first (1873) produced an endocarditis in lower animals by mechanical injury to the cardiac valves, effected by introducing a sound through the aorta. Following his method, Wyssokowitsch (1885), after injuring the cardiac valves in rabbits, injected into the circulation pure cultures of various bacteria. He obtained positive results with Staphylococcus aureus and Streptococcus pyogenes only. When these micrococci were injected into the trachea or subcutaneously the result was negative, as was the case when very few cocci were injected into a vein, or when two days or more were allowed to elapse after injury to the cardiac valves. Subsequently Weichselbaum, Prudden, and Fränkel and Sänger obtained confirmatory results, thus establishing the fact that when the valves are first injured mechanically (or chemically—Prudden) the injection into a vein of a pure culture of Staphylococcus aureus gives rise to a genuine ulcerative endocarditis. It has been further shown by Ribbert that the same result may be obtained without previous injury to the valves by injecting into a vein the staphylococcus from a potato culture suspended in water. In his experiments not only the micrococci from the surface but the superficial layer of the potato was scraped off with a sterilized knife and mixed with distilled water; and the successful result is ascribed to the fact that the little agglomerations of micrococci and infected fragments of potato attach themselves to the margins of the valves more readily than isolated cocci would do. In these experiments the mitral and tricuspid valves were affected, while the semilunar valves remained intact. In ulcerative endocarditis it is evident that cocci detached from the diseased valves must find their way into the circulation. As a matter of fact, masses of micrococci are carried away by the blood stream and form emboli in various parts of the body, which become secondary foci of infection and give rise to local necrotic changes and accumulations of pus. While this undoubtedly occurs, it is generally admitted that the mycotic infection of the cardiac valves is usually a secondary affection, resulting from the transportation of micrococci in the blood current from some other infected focus. But there is no general development of micrococci in the circulating fluid, and in man, as in animals infected experimentally, a microscopic examination of the blood for microörganisms usually gives a negative result. Culture experiments may, however, demonstrate their presence. Thus recent investigations by Netter, Eiselberg, and others show that the pus cocci are usually present in the blood in small numbers, as demonstrated by culture experiments, in septic infection from wounds.

STAPHYLOCOCCUS PYOGENES ALBUS.

Isolated by Rosenbach (1884) from the pus of acute abscesses, in which it is sometimes the only microörganism present, and sometimes associated with other pus cocci. In thirty-three acute abscesses examined by Passet (1885) it was associated with Staphylococcus aureus in eleven, with Staphylococcus citreus in two, with Streptococcus pyogenes in one, with both Staphylococcus citreus and Streptococcus pyogenes in one, and was obtained alone from four.

In its *morphology* this micrococcus is identical with the preceding, but it is distinguished from it by the absence of pigment and by being somewhat less pathogenic. Surface cultures upon nutrient agar or potato have a milk-white color. It liquefies gelatin in the same way as does the golden staphylococcus, but the deposit at the bottom of the liquefied gelatin is without color. In the temperature conditions favorable to its growth, and in its biological characters generally, with the exceptions noted, it is not to be distinguished from the species previously described. According to Flügge, it is more common than aureus among many of the lower animals.

Pathogenesis.—Fortunati has tested the comparative pathogenic power of Staphylococcus aureus and Staphylococcus albus by inoculations into the cornea of rabbits. A purulent infiltration of the cornea and panophthalmitis resulted when Staphylococcus aureus was inoculated upon the surface of the cornea by scratching with an infected needle, but inoculations made in the same way with Staphylococcus albus healed spontaneously or gave rise to a perforating ulcer. After paracentesis of the cornea with an instrument infected with Staphylococcus aureus panophthalmitis developed in thirty hours; the same result occurred at the end of sixty to seventy-two hours when the instrument was infected with Staphylococcus albus. When a sterilized instrument was used the result was negative. In bacteriological researches made by Gallenga, in cases of panophthalmitis in man, Staphylococcus albus was found in ten cultures and Staphylococcus aureus in nine.

Staphylococcus Epidermidis Albus (Welch).

The researches of Welch show that a white staphylococcus, probably identical with Staphylococcus pyogenes albus of Rosenbach, is the most common microörganism upon the surface of the body, and that "it is very often present in parts of the epidermis deeper than can be reached by any known means of cutaneous disinfection save the application of heat." With reference to this coccus Welch says:

> "So far as our observations extend—and already they amount to a large number—this coccus may be regarded as a nearly, if not quite, constant inhabitant of the epidermis. It is now clear why I have proposed to call it the Staphylococcus epidermidis albus. It possesses such feeble pyogenic capacity, as is shown by its behavior in wounds as well as by experiments on rabbits, that the designation Staphylococcus pyogenes albus does not seem appropriate. Still, I am not inclined to insist too much upon this point, as very probably this coccus, which has hitherto been unquestionably identified by Bossowski and others with the ordinary Staphylococcus pyogenes albus of Rosenbach, is an attenuated or modified form of the latter organism, although, as already mentioned, it presents some points of difference from the classical description of the white pyogenic coccus."

According to Welch, this coccus differs from Staphylococcus pyogenes aureus not only in color, but also in the fact that it liquefies gelatin more slowly, does not so quickly cause coagulation of milk, and is far less virulent when injected into the circulation of rabbits. It has been shown by the researches of Bossowski and of Welch that this coccus is very frequently present in aseptic wounds, and that usually it does not materially interfere with the healing of wounds, although sometimes it appears to cause suppuration along the drainage tube, and it is the usual cause of "stitch abscess." Bossowski, in fifty cases of wounds treated antiseptically, obtained bacteria from the discharges in forty, and in twenty-six of these cases he found Staphylococcus pyogenes albus; Staphylococcus aureus was found nine times, Streptococcus pyogenes in two, and various non-pathogenic bacteria in eight. In forty-five laparotomy wounds examined by Ghrisky and Robb, in which strict antiseptic precautions had been observed, bacteria were found in thirty-one, and in nineteen of this number Staphylococcus albus was present, Staphylococcus aureus in five, Bacillus coli communis in six, and Streptococcus pyogenes in three.

STAPHYLOCOCCUS PYOGENES CITREUS.

Isolated by Passet (1885) from the pus of acute abscesses. In thirty-three cases examined it was found associated with Staphylococcus albus in two and with Staphylococcus albus and Streptococcus pyogenes in one.

In its *morphology* this coccus is identical with the two preceding species, from which it is distinguished by the formation of a lemon-yellow pigment, instead of a golden or orange-yellow as in Staphylococcus aureus. The pigment is only formed in the presence of oxygen. This coccus is said by Fränkel to liquefy gelatin more slowly than the previously described species —Staphylococcus aureus and Staphylococcus albus.

As to its *pathogenic properties* we have no definite information. It is included among the pyogenic bacteria because of its occasional presence in the pus of acute abscesses, although it has heretofore only been found in association with other microörganisms.

MICROCOCCUS PYOGENES TENUIS.

Obtained by Rosenbach (1884) from pus in three cases out of thirty-nine examined.

Morphology.—Micrococci, somewhat irregular in size, but larger than Staphylococcus albus, and seldom associated in masses. Frequently the individual cocci present the appearance of consisting of two deeply stained masses separated from each other by a paler interspace. Cultures upon the surface of nutrient agar form a very thin, transparent layer of about one millimetre in breadth along the line of inoculation; this resembles a thin layer of varnish.

Pathogenesis undetermined. (Micrococcus pneumoniæ crouposæ ?)

STREPTOCOCCUS PYOGENES.

Synonyms.—Micrococcus of erysipelas (Fehleisen) ; Streptococcus erysipelatos ; Streptococcus of pus ; Streptococcus longus (Von Lingelsheim).

Obtained by Fehleisen from the skin involved in cases of erysipelas (1883), and by Rosenbach (1884) and Passet (1885) from the pus of acute abscesses. The characters of the "streptococcus of erysipelas" of Fehleisen and the "Streptococcus pyogenes" of Rosenbach and Passet are generally admitted to be identical, although some bacteriologists still describe them separately and cultures from the two sources are still retained in bacteriological laboratories under the names originally given them.

Rosenbach found Streptococcus pyogenes alone in fifteen cases, and associated with staphylococci in five cases, out of thirty-nine cases examined of acute pus formation. Passet, in thirty-three similar cases, obtained the streptococcus alone in eight and associated with staphylococci in two. Subsequent researches show that this micrococcus is frequently, if not constantly, present in puerperal metritis ; that it is the most frequent microörganism associated with ulcerative endocarditis ; that it is frequently present in diphtheritic false membranes, and especially in those cases of diphtheritic inflammation which are secondary to scarlet fever and measles (Prudden). Numerous investigations made by bacteriologists during the past few years indicate that this is a very important and widely distributed pathogenic microörganism. It has also been frequently found upon exposed mucous surfaces—mouth, nose, vagina—of healthy individuals.

According to the researches (1891) of Von Lingelsheim, the Streptococcus pyogenes differs from Streptococcus erysipelatos in being pathogenic both for mice and rabbits, while the latter is pathogenic for rabbits only. The author named, as a result of extended and carefully conducted comparative studies, arrives at the following conclusions:

> "According to my observations, there are two great groups among the streptococci. These cannot be distinguished one from the other in cultures in highly albuminous media (pus, blood serum), but present constant differences when cultivated in bouillon. The decisive characteristics in this medium are : macroscopic, the cloudiness of the medium ; microscopic, the length of the chains. The two groups are with difficulty distinguished in agar cultures; more easily in gelatin, in which the streptococcus which forms short chains causes a slight liquefaction, while the Streptococcus longus does not. Upon potato Streptococcus brevis alone shows a visible growth. . . . We see here a group of streptococci which we separate from the others, because of their microscopic and cultural differences, under the name of Streptococcus brevis, which is also distinguished by having no pathogenic action upon the animals usually experimented upon. We recognize, on the other hand, the streptococci which we have grouped together as Streptococcus longus as all pathogenic and about in equal degree for a certain species of animal (rabbits); but by experiments upon other species (mice) we arrive at the conclusion that there must also be differences between these streptococci. It appears that the streptococci which are distinguished by their high degree of pathogenic power upon mice are also those which are distinguished in bouillon cultures by the formation of conglomerate masses. We find among these also one which is distinguished by especial virulence for mice, and that this one is distinguished in cultures by its scanty growth upon ox serum."

The more recent researches of Knorr (1893), and of Waldvogel (1894), indicate that the classification of the streptococci proposed by von Lingelsheim has no great value, and show that marked changes in biological characters and in pathogenic power may result from cultivation in special media, or from successive inoculations into animals.

Morphology.—Spherical cocci, from 0.4 μ to 1 μ in diameter, but varying considerably in dimensions in different cultures, and even in a single chain. Multiply by binary division, in one direction only, forming chains, in which the elements are commonly associated in pairs. Under certain circumstances, instead of forming chains, a culture may contain only, or chiefly, diplococci ; but usually chains containing from four to twenty or more elements are formed, and these are frequently associated in tangled masses. Occasionally one or more cells in a chain greatly exceed their fellows in size, and some bacteriologists suppose that these cells serve as reproductive spores—arthrospores—but this has not been definitely proven.

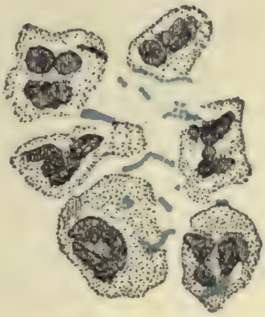

FIG. 82.—Pus containing streptococci. × 800. (Flügge.)

Stains readily with the aniline colors and by Gram's method.

Biological Characters.—Grows readily in various liquid and solid culture media, including all of those usually employed in bacteriological researches. The most favorable temperature for its development is from 30° to 37° C., but it multiplies freely at the ordinary room temperature—16° to 18° C.

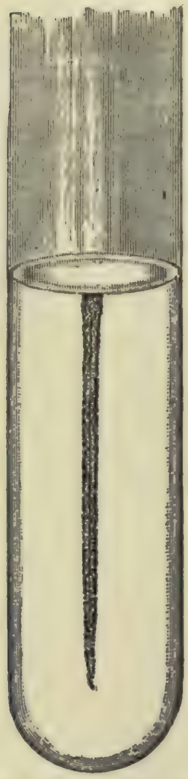

FIG. 83.—Streptococcus of erysipelas in nutrient gelatin; stick culture at end of four days at 16°-18° C. (Baumgarten).

Streptococcus pyogenes is a *facultative anaërobic*, growing both in the presence and absence of oxygen. It *does not liquefy* gelatin, and in gelatin stab cultures it grows along the line of puncture, forming numerous small, spherical, translucent, whitish colonies, which are closely crowded together at the upper portion of the line of growth, and often distinctly separated from each other below; upon the surface there is often no growth, or a scanty development may occur about the point of entrance of the inoculating needle. The minute colonies along the line of puncture are already visible at the end of twenty-four hours in cultures kept in the incubating oven at 30° to 35° C., and at the end of three or four days they have reached their full development, forming a semi-opaque, white, granular column, upon the margins of which the separate colonies are seen projecting into the gelatin. On gelatin plates very small, translucent colonies are developed, which upon the surface spread out to form a flat, transparent disc of about one-half millimetre. Under a low magnifying power these colonies are seen to be slightly granular and have a yellowish color. At a later date they become darker and less transparent, and the margin may show irregular projections made up of tangled masses of cocci in chains. The characters of growth in nutrient agar and in jellified blood serum are similar to those in gelatin, and on agar plates colonies are formed similar to those above described, except that they are somewhat smaller and more transparent. Fehleisen and De Simone state that the erysipelas coccus may develop upon the surface of cooked potato, but most authorities —Flügge, C. Fränkel, Passet, Baumgarten—agree that no growth occurs upon potato. Milk is a favorable medium for the growth of this micrococcus, and the casein is coagulated by it. A slightly acid reaction of the culture medium does not prevent its development. The *thermal death-point*, as determined by the writer, is between 52° and 54° C., the time of exposure being ten minutes. According to De Simone, a temperature of 39.5° to 41° C. maintained for two days is fatal to this micrococcus.

Manfredi and Traversa have injected filtered cultures into frogs, guinea-pigs, and rabbits for the purpose of ascertaining if any soluble toxic substance is produced during the growth of Streptococcus pyogenes. They report that in some cases convulsions and in others paralysis resulted from these injections.

Von Lingelsheim has (1891) reported the following results obtained in an extended series of experiments made to determine the germicidal power of various chemical agents as tested upon this microörganism—time of exposure two hours : Hydrochloric acid 1 : 250, sulphuric acid 1 : 250, caustic soda 1 : 130, ammonia 1 : 25, mercuric chloride 1 : 2,500, sulphate of copper 1 : 200, chloride of iron 1 : 500, terchloride of iodine 1 : 750, peroxide of hydrogen 1 : 50, carbolic acid 1 : 300, cresol 1 : 250, lysol 1 : 300, creolin 1 : 130, naphthylamin 1 : 125, malachite green 1 : 3,000, pyoktanin 1 : 700.

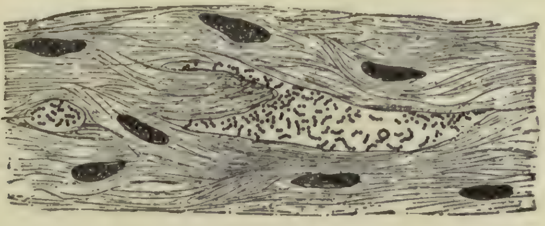

FIG. 84.—Section from margin of an erysipelatous inflammation, showing streptococci in lymph spaces. From a photograph by Koch. × 900.

Pathogenesis.—When inoculated into the cornea of rabbits Streptococcus pyogenes gives rise to keratitis. Inoculations into the ear of the same animal usually give rise to a localized erysipelatous inflammation accompanied by an elevation of temperature in the inoculated ear ; at the end of thirty-six to forty-eight hours the inflamed area, which has well-defined margins and a bright-red color, extends from the point of inoculation along the course of the veins to the root of the ear. This appearance passes away in the course of a few days and the animal recovers. Subcutaneous injections into mice or rabbits are usually without result, and the last-named animal also withstands injections of considerable quantities into the general circulation through a vein. When, however, the animal has previously been weakened by the injection of toxic substances the streptococcus may multiply in its body and cause its death (Flügge).

Fehleisen has inoculated cultures, obtained in the first instance from the skin of patients with erysipelas, into patients in hospital suffering from lupus and carcinoma, and has obtained positive results, a typical erysipelatous inflammation having developed around the point of inoculation after a period of incubation of from fifteen to sixty hours. This was attended with chilly sensations and an elevation of temperature. Persons who had recently recovered from an attack of erysipelas proved to be immune.

Sections made from the ear of an inoculated rabbit, or of skin taken from the affected area in erysipelas in man, show the streptococci in considerable numbers in the lymph channels, but not in the blood vessels. They are more numerous, according to Koch and to Fehleisen, upon the margins of the erysipelatous area, and may even be seen in the lymph channels a little beyond the red margin which marks the line of progress of the infection.

The researches of Weichselbaum and others show that Streptococcus pyogenes is the infecting microörganism in a certain proportion of the cases of *ulcerative endocarditis.* The author named found it in four cases out of fifteen examined, and in two cases of endocarditis verrucosa out of thirteen. In a previously reported series of sixteen cases (fourteen of ulcerative endocarditis and two of verrucosa) the streptococcus was found in six.

In *diphtheritic false membranes* this streptococcus is very commonly present, and in certain cases attended with a diphtheritic exudation, in which the Bacillus diphtheriæ has not been found by competent bacteriologists, it seems probable that Streptococcus pyogenes is the pathogenic microörganism responsible for the local inflammation and its results. Thus in a series of twenty-four cases studied by Prudden in 1889 the bacillus of Löffler was not found, "but a streptococcus apparently identical with Streptococcus pyogenes was found in twenty-two." Chantemesse and Widal have also reported cases in which a fibrinous exudate resembling that of diphtheria was associated with a streptococcus. "These forms of so-called diphtheria are most commonly associated with scarlatina and measles, erysipelas, and phlegmonous inflammation, or occur in individuals exposed to these diseases; but whether exclusively under these conditions is not yet established" (Prudden).

Löffler has described under the name of *Streptococcus articulorum* a micrococcus obtained by him from the affected mucous membrane in cases of diphtheria, and which he believes to be accidentally present and without any etiological import in this disease. In its characters it closely resembles Streptococcus pyogenes and is perhaps a variety of this widely distributed species. Its characters are described by Flügge as follows:

"Cultivated in nutrient gelatin, it forms at the end of three days small, transparent, light-gray drops, upon the margin of which, under the microscope, the cocci in twisted chains may be observed. As many as one hundred elements may be found in a single chain, and some of these are distinguished by their size; occasionally whole chains are made up of these large cocci, and when closely observed some of these may present indications of division transversely to the axis of the chain. Subcutaneous inoculation of cultures into mice results in the death of a considerable number of these animals—more than half; and the streptococci are found in the spleen and other organs. Inoculation into the ear of rabbits causes an erysipelatous inflammation. When injected into the circulation of these animals through a vein joint affections are developed in from four to six days, and a purulent accumulation occurs in which the streptococci are found. In two rabbits inoculated in the same way with a culture of the streptococcus of erysipelas, Löffler has observed a similar result."

Numerous researches indicate that infection by Streptococcus pyogenes through the endometrium is the usual cause of *puerperal fever.* Thus Clivio and Monti demonstrated its presence in five cases of puerperal peritonitis. Czerniewski found it in the lochia of a large number (thirty-five out of eighty-one) of women suffering from puerperal fever, but in the lochia of fifty-seven healthy puerperal women he was only able to find it once. In ten fatal cases he found it in every instance, both in the lochial discharge during life and in the organs after death. Widal carefully studied a series of sixteen cases and arrived at the conclusion that this was the infecting microörganism in all. Bumm and other observers have given similar evidence. Eiselsberg and Emmerich have succeeded in demonstrating the presence of the streptococcus in hospital wards containing cases of erysipelas. That puerperal fever may result from infection through the finger of the accoucheur, when he has previously been in contact with cases of erysipelas, has long been taught, and, in view of the facts above recorded, is not difficult to understand. But in view of the fact that the streptococcus of pus has been found in vaginal mucus and in the buccal and nasal secretions of healthy persons, it may appear strange that cases of puerperal fever not traceable to infection from erysipelas or from preceding cases do not occur more frequently. This is probably largely due to an attenuation of the pathogenic power of the streptococcus when it leads a saprophytic existence. Widal asserts that, when cultivated in artificial media for a few weeks, the cultures no longer have their original virulence, and Bumm has made the same observation. On the other hand, in "streptococcus-peritonitis" occurring as a result of puerperal infection Bumm states that the thin, bright-yellow, odorless fluid contained in the cavity of the abdomen is extremely virulent; a very slight trace, a fragment of a drop, injected into the abdominal cavity of a rabbit, is sufficient within twenty-four hours to cause a general septic inflammation with a bloody serous exudation, quickly terminating in the death of the animal; injected subcutaneously it gives rise to an enormous phlegmon which also quickly proves fatal. But cultures of Streptococcus pyogenes, after it has been carried through successive generations in artificial media, injected beneath the skin of a rabbit, usually produce no result, or at most an abscess of moderate dimensions.

It seems probable that the micrococcus isolated by Flügge from necrotic foci in the spleen of a case of leucocythæmia, and described by him under the name of Streptococcus pyogenes malignus, was simply a very pathogenic variety of the streptococcus of pus. He was not able to differentiate it from Streptococcus pyogenes by its morphology or growth in culture media, but it proved far more pathogenic when tested upon animals. Mice inoculated subcutaneously with a minute quantity of a pure culture died, without exception, in three to five days. A large abscess was formed at the point of inoculation, and the blood of the animal contained numerous cocci in pairs and chains. Rabbits inoculated in the ear showed at first the same local appearances as result from inoculations with streptococcus of pus and of erysipelas, but after two or three days symptoms of general infection were developed, and death occurred at the end of three or four days. At the autopsy the cocci were found in the blood, and frequently there were purulent collections in the joints containing the same microörganism. Krause has also described a streptococcus which only differs from Streptococcus pyogenes of Rosenbach and Passet by the greater virulence manifested by its cultures.

The fact that pathogenic bacteria may attain an intensified degree of virulence by cultivation in the bodies of susceptible animals was demonstrated by Davaine many years ago, and is fully established by the experiments of Pasteur and others. It is true of the anthrax bacillus, of the writer's Micrococcus Pasteuri, and of other well-known pathogenic microörganisms. The reverse of this—attenuation of virulence as a result of cultivation in artificial media—is also well established for several pathogenic species. Now it appears that the attenuated streptococcus is far less likely to give rise to erysipelas or to puerperal infection than is the same microörganism as obtained from a case of one or the other of these infectious diseases. The same is probably true also of Staphylococcus aureus and other facultative parasites which are found as saprophytes upon the surface of the body and upon exposed mucous membranes in healthy persons. And it is not improbable that attenuated varieties of these micrococci which find their way into open wounds, or into the uterine cavity shortly after parturition, if they escape destruction by the sanguineous discharge, acquire increased pathogenic power from their multiplication in it, as a result of which they are able to invade the living tissues. But it appears probable that infection through open wounds does not depend alone upon the potency of the pathogenic micrococci present in them, but also upon the absorption of chemical poisons produced by septic (putrefactive) bacteria, which weaken the vital resisting power of the tissues. Gottstein, as a result of experiments made by him, is of the opinion that the resorption of broken-down red blood corpuscles favors infection by pathogenic bacteria present in wounds ; and he has shown that the injection into animals of certain toxic substances which destroy the red corpuscles in the circulation makes them susceptible to the pathogenic action of certain bacteria which are harmless for them under ordinary circumstances. Thus a guinea-pig, an animal which is immune against the bacillus of fowl cholera, succumbed to an inoculation made after first injecting subcutaneously 0.06 gramme of hydracetin dissolved in alcohol. At the autopsy hæmorrhagic exudations were found in the serous cavities, hæmorrhagic infarctions in the lungs, and quantities of the bacillus injected were found in the blood and in fluid from the cavity of the abdomen.

In man the ever-present pus cocci are more likely to invade the tissues, forming furuncles, carbuncles, and pustular skin eruptions, or erysipelatous and phlegmonous inflammations, when the standard of health is reduced from any cause, and especially when by absorption or retention various toxic organic products are present in the body in excess. It is thus that we would explain the liability to these local infections, as complications or sequelæ of various specific infectious diseases, in the victims of chronic alcoholism, in those exposed to septic emanations from sewers, etc., and probably in many cases from the absorption of toxic products formed in the alimentary canal as a result of the ingestion of improper food, or of abnormal fermentative changes in the contents of the intestine, or from constipation.

The Pus Cocci in Inflammations of Mucous Membranes.—To what extent the pus cocci are responsible for inducing and maintaining non-specific inflammations of mucous membranes has not been determined ; but having demonstrated the pyogenic properties of these cocci, their presence in the purulent discharges from inflamed mucous membranes can scarcely be considered as unimportant, notwithstanding the fact that they are also frequently found in secretions from healthy mucous surfaces. They are likewise found upon the skin of healthy persons, and yet we have unimpeachable experimental evidence that they may produce a local inflammation, attended with pus formation, when injected subcutaneously, or even when freely applied to the uninjured surface.

In *otitis media* Levy and Schrader obtained Staphylococcus albus in pure cultures in three cases out of ten in which paracentesis was performed, and in two others it was present in association with other microörganisms. In eighteen cases of otitis media in young children Netter found Staphylococcus aureus six times and Streptococcus pyogenes thirteen times. Scheibe, in eleven cases in which perforation had not yet taken place, found Staphylococcus albus in two and various other microörganisms in the remaining cases ; Staphylococcus aureus was not present in any. Habermann obtained aureus associated with other bacteria in a single case of purulent otitis media. In a series of eight cases occurring as a sequela of influenza Scheibe obtained Streptococcus pyogenes in two, "diplococcus pneumoniæ" in two, Staphylococcus aureus in one, Streptococcus pyogenes and Staphylococcus albus together in two, and Streptococcus pyogenes in association with an undescribed micrococcus in one. In all of these cases a slender bacillus was also present, as shown by microscopical examination, which did not grow in any of the culture media employed. Bordoni-Uffreduzzi and Gradenigo have tabulated the results obtained by various bacteriologists who have examined pus obtained through the previously intact tympanic membrane. In thirty-two cases of this character the microörganism most frequently found was diplococcus pneumoniæ (Micrococcus pneumoniæ crouposæ of the present writer), which was present in a pure culture in thirteen and associated with Staphylococcus aureus in one, with Staphylococcus albus in one, and with Streptococcus pyogenes in one. In the other sixteen cases the pyogenic cocci were present in all but two, in which bacilli were found—Bacillus tenuis in one, a non-liquefying bacillus in one. In twenty-seven cases in which the pus was withdrawn from one to thirty days after paracentesis or spontaneous rupture of the membrane, the pyogenic cocci were present in twenty and diplococcus pneumoniæ in seven.

In acute *nasal catarrh* Paulsen found Staphylococcus aureus in seven cases out of twenty-four examined, and E. Fränkel in two out of four ; but it must be remembered that Von Besser has shown that this micrococcus is frequently present in the secretions from the healthy nasal mucous membrane, and we have experimental evidence that the pus organisms, when introduced into the conjunctival sac of rabbits (Widmark), do not give rise to catarrhal inflammation. On the other hand, Widmark found that when inoculated into the cornea of rabbits an intense *conjunctivitis* resulted, together with keratitis and perforation of the cornea in fifteen per cent of the cases. The same author in his bacteriological researches obtained the pyogenic staphylococci from the circumscribed abscesses of blepharadenitis, while in inflammation of the lacrymal sac Streptococcus pyogenes was usually present.

Shougolowicz, in the bacteriological examination of twenty-six cases of *trachoma*, found Staphylococcus albus in twelve, Staphylococcus aureus in nine, Staphylococcus citreus in three, and Staphylococcus cereus albus in three. These pus organisms were in a number of the cases associated with other well-known saprophytes, and in seven cases a short bacillus not previously described was found. That various bacilli are found in the conjunctival sac of healthy eyes and in different forms of conjunctivitis has been shown by Fick, whose results do not correspond in this respect with those of Gifford, who found almost exclusively micrococci. Whatever may be the final conclusion as to the rôle of the pus cocci heretofore described in the etiology of acute or chronic conjunctivitis, there can be no doubt of the power of the "gonococcus" to induce a virulent inflammation of the conjunctivæ when introduced into healthy eyes.

MICROCOCCUS GONORRHŒÆ.

Synonym.—Gonococcus (Neisser).

Discovered by Neisser (1879) in gonorrhœal pus and described by him under the name of "Gonococcus." Cultivated by Bumm (1885), and infective virulence proved by inoculation into man. Constantly present in virulent gonorrhœal discharges, for the most part in the interior of the pus cells or attached to the surface of epithelial cells.

Morphology.—Micrococci, usually joined in pairs or in groups of four, in which the elements are flattened — "biscuit-shaped." The flattened surfaces face each other and are separated, in stained preparations, by an unstained interspace. The diameter of an associated pair of cells varies from 0.8 to 1.6 μ in the long diameter—average about 1.25 μ—and from 0.6 to 0.8 μ in the line of the interspace between the biscuit-shaped elements, which sometimes present a slight concavity of the flattened surfaces. Multiplication occurs alternately in two planes, and as a result of this groups of four are frequently observed. But diplococci are more numerous and are considered as the characteristic mode of grouping. Single, spherical, undivided cells are rarely seen.

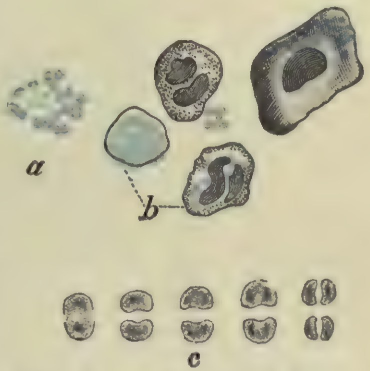

FIG. 85.—*a*, gonococci from a pure culture, × about 1,000; *b*, gonococci in pus cells and epithelial cell from case of gonorrhœal ophthalmia; *c*, form and mode of division of gonococci—schematic. (Bumm.)

It must be remembered that the morphology of this micrococcus as above described does not suffice to distinguish it, for Bumm has shown that "the biscuit form is not at all specific for the gonococcus, but is shared with it by a number of microörganisms, which consist of two hemispherical elements with the flattened surfaces facing each other and separated by a cleft, and some of these correspond in their morphology, in every detail, with the gonococcus."

Stains quickly with the basic aniline colors, especially with methyl violet, gentian violet, and fuchsin; not so quickly with methylene blue, which is, however, one of the most satisfactory staining agents for demonstrating its presence in pus. Beautiful double-stained preparations may be made from gonorrhœal pus, spread upon a cover glass and "fixed," *secundum artem*, by the use of methylene blue and eosin. Does not stain by Gram's method—*i.e.*, the cocci are decolorized, after having been stained with an aniline color, by being immersed in the iodine solution employed in Gram's method of staining. But this character cannot be depended upon alone for establishing the diagnosis, for Bumm has shown that other diplococci are occasionally found in gonorrhœal pus which do not stain by this method. It serves to distinguish them, however, from the common pus cocci heretofore described—Staphylococcus aureus, Staphylococcus albus, Staphylococcus citreus—which retain their color when treated in the same way. A more trustworthy diagnostic character is that these biscuit-shaped diplococci are found within the pus cells, sometimes one or two pairs only, but more frequently in considerable numbers, and occasionally in such numbers as to completely fill the cell. No similar picture is presented by pus from any other source, with the exception of that from a form of "puerperal cystitis" which Bumm has described. But in this the diplococci contained in the pus cells were to be distinguished by the fact that they retained their color when treated by Gram's method. Owing

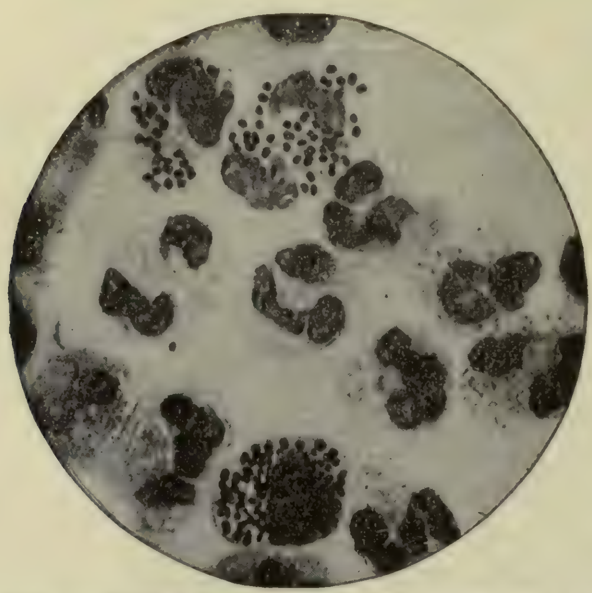

FIG. 86.—"Gonococcus" in gonorrhœal pus. From a photomicrograph by Fränkel and Pfeiffer. × 1,000.

to the difficulty of cultivating this micrococcus, and the importance, under certain circumstances, of not making a mistake in its diagnosis, these characters are of exceptional value.

Biological Characters.—Bumm (1885) first succeeded in cultivating the "gonococcus" upon human blood serum, obtained from the placenta of a recently delivered woman. He found that the cultures thrive best in a moist atmosphere at 30° to 34° C. The growth under the most favorable conditions is slow, and frequently no development occurs when pus containing numerous gonococci is placed upon blood serum in an incubating oven; or after a slight multiplication development ceases and the cocci undergo degenerative changes and quickly disappear.

Cultures upon the surface of blood serum form a very thin, often scarcely visible layer, with a smooth, moist, shining surface, and by reflected light a grayish-yellow color. The growth at the end of twenty-four hours may extend for a distance of a millimetre along the line of inoculation, but at the end of two or three days no further development occurs and the cocci soon lose their vitality. This micrococcus, then, is *aërobic.* Whether it may also be a facultative anaërobic has not been definitely determined, but it does not grow along the line of puncture when stick cultures are made in blood serum. Its rapid and abundant multiplication in gonorrhœal infection of mucous membranes, and the difficulties attending its cultivation in artificial media, show that the gonococcus is a *strict parasite.*

Lestikow and Löffler, prior to the publication of Bumm's important monograph, had reported successful results in cultivating the gonococcus upon a mixture of blood serum and gelatin. Bockhart has since recommended a mixture of nutrient agar (two parts), liquefied at a temperature of 50° C., with blood serum (two to three parts) at 20° C. By quickly mixing with this a little pus containing the gonococcus he was able to obtain colonies upon plate cultures, made by pouring the liquid medium upon sterile glass plates in the usual manner.

Ghon and Schlagenhaufer in 1893 reported that they obtained good results by adding phosphate of soda to blood-serum agar, made according to the method of Wertheim—one part of human blood serum from the placenta to two or three parts of nutrient agar. Also that they were successful in cultivating the gonococcus in an acid medium made by adding one part of urine to two of nutrient agar (two per cent). Turro (1894) has since published the results of his experiments relating to the cultivation of this micrococcus in acid media. According to him it grows in normal urine, either with or without the addition of peptone (one per cent); also in acid gelatin, prepared in the usual way but without neutralization (?).

Turro also claims to have produced specific urethritis in dogs by inoculation with his cultures. Heiman (1895) as a result of an extended experimental research, arrives at the conclusion that "the diplococcus described by Turro in connection with his acid media is not the gonococcus." His inoculation experiments in dogs, made with pure cultures of the gonococcus, gave an entirely negative result. For the cultivation of the gonococcus, Heiman recommends a medium made from "chest serum" obtained from a patient suffering with hydrothorax or acute pleurisy. This was found to be superior to placenta serum, sheep-blood serum, or peritoneum serum, because of the great amount of serum albumin which it contains. Two per cent of agar, one per cent of peptone, and one-half per cent of sodium chloride were added to the chest serum, and the medium was sterilized by "fractional sterilization."

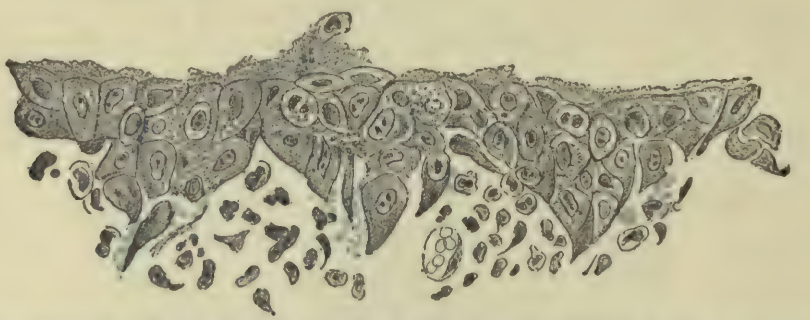

FIG. 87.—Gonorrhœal conjunctivitis, second day of sickness; section through the mucous membrane of upper eyelid; invasion of the epithelial layer by gonococci. (Bumm.)

Schrötter and Winkler (1890) report their success in cultivating the gonococcus upon albumin from the egg of the pewit—"*Kibitz.*" In the culture oven at 38° C. a thin, transparent, whitish layer was already visible at the end of six hours and rapidly extended; the growth was less abundant at the end of three days, and had entirely ceased by the fifth day. Attempts to cultivate the same microörganism in albumin from hens' eggs gave a negative result.

Aufuso (1891) has cultivated the gonococcus in fluid obtained from the knee joint in a case of chronic synovitis, but failed to cultivate it in the fluid of ascites. A culture of the twelfth generation made upon the culture medium mentioned, solidified by heat, was introduced into the urethra of a healthy man and gave rise to a characteristic attack of gonorrhœa.

Development does not occur below 25° or above 38° C. The writer has shown that a temperature of 60° C. maintained for ten minutes destroys the infective virulence of gonorrhœal pus.

Pathogenesis.—That the gonococcus is the cause of the specific inflammation and purulent discharge characteristic of gonorrhœa is now generally admitted upon the experimental evidence obtained by

Bumm. Having succeeded in obtaining it in pure cultures from gonorrhœal pus, he made successful inoculations in the healthy urethra in two cases—once with a third culture and once with one which had been transferred through twenty successive generations. In both cases a typical gonorrhœa developed as a result of the inoculation.

The mucous membranes in man which are subject to gonorrhœal infection are those of the urethra, the conjunctiva, the cervix uteri, and the vagina in children—the vagina in adults is not involved. Inoculations of gonorrhœal pus into the vagina or conjunctival sac of the lower animals—dogs, rabbits, horses, apes—are without result.

The very numerous researches which have been made by competent bacteriologists show that the gonococcus is constantly present in gonorrhœal discharges, and in view of the facts above stated its etiological import appears to be fully established. Bumm has studied the development of blennorrhœa neonatorum, and has shown that soon after infection the presence of gonococci may be demonstrated in the superficial epithelial cells of the mucous membrane and between them ; that they soon penetrate to the deeper layers, and that by the end of forty-eight hours the entire epithelial layer is invaded by the diplococci, which penetrate by way of the connecting material—" *Kittsubstance* "—between the cells. They also multiply in the superficial layers of connective tissue and give rise to an inflammatory reaction, which is shown by an abundant escape of leucocytes from the dilated capillary network. The penetration of the gonococci to the deeper layers of the mucous membrane of the urethra, and even to the corpus cavernosum, was observed by Bockhart in a case studied by him in which death occurred during an acute attack of gonorrhœa. But Bumm concludes from his researches that this is not usual, and that the invasion is commonly limited to the superficial layers of the mucous membrane.

Staphylococcus pyogenes aureus is not infrequently associated with the gonococcus in late gonorrhœal discharges, and the abscesses which occasionally develop as a complication of gonorrhœa, in the prostate, the inguinal glands, or around the urethra, are probably due to its presence, which has been demonstrated in the pus from such abscesses in a number of cases. The same is true of the joint affections and endocarditis which sometimes occur in the course of an attack of gonorrhœa. Although some authors have claimed to find the gonococcus in these so-called metastatic gonorrhœal inflammations, the evidence is not satisfactory, and it seems probable that the Staphylococcus aureus is the usual microörganism concerned in these affections.

VI.

BACTERIA IN CROUPOUS PNEUMONIA.

BACILLUS OF FRIEDLÄNDER.

Synonyms.—Pneumococcus (Friedländer); Bacillus pneumoniæ (Flügge).

Obtained by Friedländer and Frobenius in pure cultures (1883) from the exudate into the pulmonary alveoli in cases of croupous pneumonia. Subsequent researches show that it is only present in a small proportion of the cases—nine times in one hundred and twenty-nine cases examined by Weichselbaum, three times in seventy cases examined by Wolf.

Morphology.—Short rods with rounded ends, often so short as to resemble micrococci, especially in very recent cultures; commonly united in pairs or chains of four, and under certain circumstances surrounded by a transparent capsule. The gelatinous envelope—so-called capsule—is not seen in preparations made from cultures in artificial media, but is very prominent in properly stained preparations from the blood of an inoculated animal. It often has a diameter equal to or greater than that of the enclosed cell, and appears to consist of a substance resembling mucin, which is soluble in water or dilute alcohol. Where several cells are united in a chain they may all be enclosed in a common envelope, or each may have its own capsule. This capsule is not peculiar to Friedländer's bacillus, as he at first supposed, but is found in other bacilli and also in the writer's Micrococcus Pasteuri.

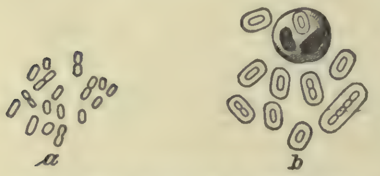

Fig. 88.—Bacillus of Friedländer; *a*, **from a culture;** *b*, **from blood of mouse, showing capsule. (Flügge.)**

Friedländer's bacillus *stains* readily with the aniline colors, but is decolorized by the iodine solution used in Gram's method. In preparations from the blood of an inoculated animal, stained by an aniline color, the capsule appears as an unstained envelope surrounding the stained cell, but by special treatment the capsule may also be stained. Friedländer's method is as follows: The section or cover-glass preparation is placed for twenty-four hours in a solution of gentian violet and acetic acid, containing fifty parts of a concentrated alcoholic solution of gentian violet, one hundred parts of distilled water, and ten parts of acetic acid. The stained preparation is washed for a minute or two in a one-per-cent solution of acetic acid, dehydrated with alcohol, cleared up with oil of cloves or cedar, and mounted in balsam. The bacillus is quickly stained in dried cover-glass preparations by immersion in aniline-water-gentian-violet solution (two or three minutes). The stained preparation should be decolorized by placing it in absolute alcohol for half a minute, and then washed in distilled water.

Biological Characters.—This bacillus does not, so far as is known, form reproductive spores; it is *non-motile* and *does not liquefy gelatin.* It is *aërobic* and a *facultative anaërobic.* In gelatin stab cultures it presents the "nail-shaped" growth first described by Friedländer, which is not, however, peculiar to this bacillus. The head of the nail is formed by the development around the point of entrance of the inoculating needle of a rounded, white mass having a smooth, shining surface, and its stem by the growth along the line of puncture. This consists of closely crowded, opaque, white, spherical colonies. Gas bubbles sometimes develop in gelatin cultures, and in old cultures the gelatin about the line of growth acquires a yellowish-brown color. The growth in nutrient agar resembles that in gelatin. Upon the surface of blood serum abundant grayish-white, viscid masses are developed. Upon potato the growth is abundant, quickly covering the entire surface with a thick, yellowish-white, glistening layer which often contains gas bubbles when the temperature is favorable. Colonies in gelatin plates appear at the end of twenty-four hours as small, white spheres, which increase rapidly in size, and upon the surface form rounded, smooth, glistening, white masses of considerable size. Under the microscope the colonies present a somewhat irregular outline and a slightly granular appearance. Growth occurs at comparatively low temperatures—16° to 20° C.—but is more rapid in the incubating oven. The thermal death-point, as determined by the writer, is about 56° C. In the ordinary culture media it retains its vitality for a long time, and may grow when transplanted to fresh culture material after having been pre-

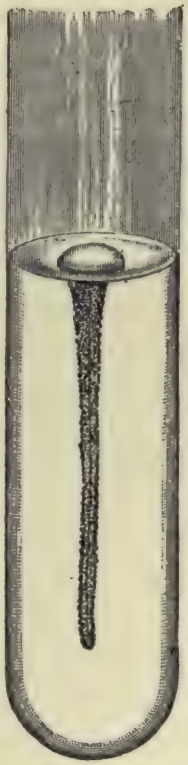

FIG. 89.—Friedländer's bacillus; stab culture in gelatin; end of four days at 16°–18° C. (Baumgarten.)

served for a year or more. At a temperature of 40° C. development ceases.

Pathogenesis.—In Friedländer's experiments the bacillus from pure cultures, suspended in water, was injected through the thoracic wall into the right lung of dogs, rabbits, guinea-pigs, and mice. Rabbits proved to be immune; one dog out of five, six guinea-pigs out of eleven, and all of the mice (thirty-two) succumbed to the inoculation. At the autopsy the pleural cavities were found to contain a sero-purulent fluid; the lungs were intensely congested, contained but little air, and in places showed limited areas of red infiltration; the spleen was considerably enlarged; the bacillus was found in great numbers in the lungs, the fluid in the pleural cavities, and in the blood obtained from the general circulation or from the various organs of the body. Similar appearances presented themselves in the case of the guinea-pigs which succumbed to the inoculation.

These results show that the bacillus under consideration is pathogenic for mice and for guinea-pigs, but they are by no means sufficient to prove that it is capable of producing a genuine croupous pneumonia in man, and it is still uncertain whether its occasional presence in the exudate into the pulmonary alveoli in cases of this disease has any etiological importance.

MICROCOCCUS PNEUMONIÆ CROUPOSÆ.

Synonyms.—Micrococcus Pasteuri (Sternberg); Micrococcus of sputum septicæmia (Fränkel); Diplococcus pneumoniæ (Weichselbaum); Bacillus septicus sputigenus (Flügge); Bacillus salivarius septicus (Biondi); Lancet-shaped micrococcus (Talamon); Streptococcus lanceolatus Pasteuri (Gameléia).

Discovered by the present writer in the blood of rabbits inoculated subcutaneously with his own saliva in September, 1880; by Pasteur in the blood of rabbits inoculated with the saliva of a child which died of hydrophobia in one of the hospitals of Paris in December, 1880; identified with the micrococcus in the rusty sputum of pneumonia, by comparative inoculation and culture experiments, by the writer in 1885 (paper published in the *American Journal of the Medical Sciences,* July 1st, 1885). Proved to be the cause of croupous pneumonia in man by the researches of Talamon, Salvioli, Sternberg, Fränkel, Weichselbaum, Netter, Gameléia, and others.

The Presence of Micrococcus Pasteuri in the Salivary Secretions of Healthy Individuals.—In September, 1880, while engaged in investigations relating to the etiology of the malarial fevers, I injected a little of my own saliva beneath the skin of two rabbits as a control experiment. To my surprise the animals died, and I found in their blood a multitude of oval microörganisms, united for the most part in pairs, or in chains of three or four elements. These experiments are recorded in my paper entitled "Experimental Investigations Relating to the Etiology of the Malarial Fevers," published in the Report of the National Board of Health for 1881, pp. 74, 75.

Following up my experiments made in New Orleans (in September, 1880), in Philadelphia (January, 1881), and in Baltimore (March, 1881), I obtained the following results:

"The saliva of four students, residents of Baltimore (in March), gave negative results; eleven rabbits injected with the saliva of six individuals in Philadelphia (in January) gave eight deaths and three negative results; but in the fatal cases a less degree of virulence was shown in six by a more prolonged period between the date of injection and the date of death. This was three days in one, four days in four, and seven days in one."

In a paper published in the *Journal of the Royal Microscopical Society* (June, 1886) I say:

"My own earlier experiments showed that there is a difference in the pathogenic potency of the saliva of different individuals, and I have since learned that the saliva of the same individual may differ in this respect at different times. Thus during the past three years injections of my own saliva have not infrequently failed to cause a fatal result, and in fatal cases death is apt to occur after a somewhat longer interval, seventy-two hours or more; whereas in my earlier experiments the animals infallibly died within forty-eight hours."

The presence of my Micrococcus Pasteuri was demonstrated in the blood of the rabbits which succumbed to the inoculations.

Claxton, in a series of experiments made in Philadelphia in 1882, injected the saliva of seven individuals into eighteen rabbits. Five of these died within five days, and nine at a later period.

Fränkel, whose first publication was made in 1885, discovered the presence of this micrococcus in his own salivary secretions in 1883, and has since made extended and important researches with reference to it. The saliva of five healthy individuals and the sputa of patients suffering from other diseases than pneumonia, injected into eighteen rabbits, induced fatal "sputum septicæmia" in three only. When he commenced his experiments his saliva was uniformly fatal to rabbits, but a year later it was without effect.

Wolf injected the saliva of twelve healthy individuals, and of three patients with catarrhal bronchitis, into rabbits, and induced "sputum septicæmia" in three.

Netter examined the saliva of one hundred and sixty-five healthy persons, by inoculation experiments in rabbits, and demonstrated the presence of this micrococcus in fifteen per cent of the number.

Vignal, in his recent elaborate paper upon the microörganisms of the mouth, says :

"Last year I encountered this microbe continually in my mouth during a period of two months, then it disappeared, and I did not find it again until April of this year, and then only for fifteen days, when it again disappeared without appreciable cause."

The Presence of Micrococcus Pneumoniæ Crouposæ in Pneumonic Sputum.—Talamon, in 1883, demonstrated the presence of this micrococcus in pneumonic sputum, described its morphological characters, and produced typical croupous pneumonia in rabbits by injecting material containing it into the lungs through the thoracic walls.

Salvioli, in 1884, demonstrated its presence in pneumonic sputum by injections into rabbits.

In 1885 the writer made a similar demonstration, and by comparative experiments showed that the micrococcus present in the blood of rabbits inoculated with the rusty sputum of pneumonia was identical with that which he had discovered in 1880 in rabbits inoculated with his own saliva.

The same year (1885) A. Fränkel made a similar demonstration, and published a paper containing valuable additions to our knowledge relating to the biological characters of this microörganism (first publication appeared July 13th, 1885).

In 1886 Weichselbaum published the results of his extended researches relating to the presence of this micrococcus in the fibrinous exudate of croupous pneumonia. He obtained it in ninety-four cases (fifty-four times in cultures) out of one hundred and twenty-nine cases examined.

Wolf (1887) found it in sixty-six cases out of seventy examined.

Netter (1887) in seventy-five per cent of his cases, and in the sputum of convalescents from pneumonia in sixty per cent of the cases examined, by inoculations into rabbits.

Gameléia (1887) in twelve fatal cases of pneumonia in which he collected material from the lungs at the post-mortem examination.

Goldenberg, whose researches were made in Gameléia's laboratory, found it in pneumonic sputum in forty consecutive cases, by inoculations into rabbits and mice.

The Presence of Micrococcus Pneumoniæ Crouposæ in Meningitis.—Numerous bacteriologists have reported finding diplococci in the pus of meningitis, and frequently the microörganisms have been fully identified as "diplococcus pneumoniæ." Thus Netter (1889), in a résumé of the results of researches made by him in twenty-five cases of purulent meningitis, reports as follows :

Thirteen cases were examined microscopically, by cultures, and by inoculations into susceptible animals ; six cases by microscopical examination and experiments on animals; and the remainder only by microscopical examination. Four of the cases were complicated with purulent otitis, six with pneumonia, three with ulcerative endocarditis. The "pneumococcus" was found in sixteen of the twenty-five cases ; in four Streptococcus pyogenes was present ; in two Diplococcus intracellularis meningitidis of Weichselbaum ; in one Friedländer's bacillus ; in one Newmann and Schäffer's motile bacillus ; in one a small curved bacillus.

In forty-five cases collected from the literature of the subject by Netter this micrococcus was present in twenty-seven, Streptococcus pyogenes in six, and the Diplococcus intracellularis meningitidis of Weichselbaum in ten.

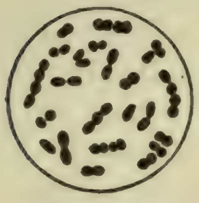

FIG. 90.

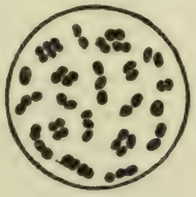

FIG. 91.

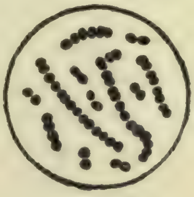

FIG. 92.

FIG. 90.—Micrococcus pneumoniæ crouposæ from blood of rabbit inoculated with normal human saliva (Dr. S.). × 1,000.

FIG. 91.—Micrococcus pneumoniæ crouposæ from blood of rabbit inoculated subcutaneously with fresh pneumonic sputum from a patient in the seventh day of the disease. × 1,000.

FIG. 92.—Surface culture of Micrococcus pneumoniæ crouposæ, on nutrient agar, showing the development of long chains. × 1,000.[1]

Monti (1889), in four cases of cerebro-spinal meningitis, demonstrated the presence of the same micrococcus. In three of his cases pneumonia was also present. In two Staphylococcus pyogenes aureus was associated with the "diplococcus pneumoniæ."

Micrococcus Pneumoniæ Crouposæ in Ulcerative Endocarditis.—Weichselbaum, in a series of twenty-nine cases examined (1888), found "diplococcus pneumoniæ" in seven.

Micrococcus Pneumoniæ Crouposæ in Acute Abscesses.—In a case of parotitis occurring as a complication of croupous pneumonia this micrococcus was obtained from the pus in pure cultures by Testi (1889); and in another case in which, as a complication of pneumonia, there developed a purulent pleuritis, abscess of the parotid on both sides, and multiple subcutaneous abscesses, the pus from all of the sources named contained the "diplococcus" in great numbers, as shown not only by microscopical examination but by inoculation into rabbits.

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[1] The above figures are from Dr. Sternberg's paper published in the American Journal of the Medical Sciences for July and October, 1885.

In a case of tonsillitis resulting in the formation of an abscess Gabbi (1889) obtained the same coccus in pure cultures.

In *otitis media* this micrococcus has been found in a considerable number of cases in the pus obtained by paracentesis of the tympanic membrane, and quite frequently in pure cultures—by Zaufal (1889) in six cases; Levy and Schrader (1889) in three out of ten cases in which paracentesis was performed; by Netter (1889) in five out of eighteen cases occurring in children.

Monti (1889) and Belfanti (1889) report cases of arthritis of the wrist joint, occurring as a complication of pneumonia, in which this micrococcus was obtained in pure cultures. Ortmann and Samter (1889), in a case of purulent inflammation of the shoulder joint following pneumonia and pleurisy, obtained the "diplococcus pneumoniæ" in pure cultures.

Morphology.—Spherical or oval cocci, usually united in pairs, or in chains consisting of three or four elements. Longer chains, containing ten or more elements, are frequently formed, especially in cultures upon the surface of nutrient agar, and in liquid media; it may therefore be regarded as a streptococcus. As observed in the blood of inoculated animals it is usually in pairs consisting of oval or lance-oval elements, which are surrounded by a transparent capsule. Owing to the elongated form of the cocci when in active growth, it has been regarded by some authors as a bacillus; but in cultures in liquid media, when development by binary division has ceased, the cells are spherical, or nearly so, and in cultures on the surface of nutrient agar the individual cells more nearly approach a spherical form than in the blood of an inoculated animal. The "lanceolate" form was first referred to by Talamon, who described it as having the form of a grain of wheat, or even still more elongated like a grain of barley, as seen in the fibrinous exudate of croupous pneumonia. The transparent material surrounding the cells—so-called capsule—is best seen in stained preparations from the fibrinous exudate of croupous pneumonia or from the blood of an inoculated animal. It appears as an unstained marginal band surrounding the elliptical cells, and varies greatly as to its extent in different preparations. This capsule probably consists of a substance resembling mucin, and, being soluble in water, its extent depends partly upon the methods employed in preparing specimens for microscopical examination. It is occasionally seen in stained preparations from the surface of cultures on blood serum; and in drop

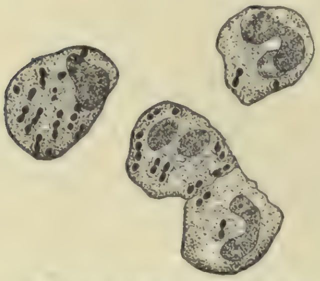

FIG. 93.—Micrococcus pneumoniæ crouposæ, showing capsule, attached to pus cells from exudate in pleural cavity of inoculated rabbit. (Salvioli.)

cultures examined under the microscope, by using a small diaphragm it may be seen to surround the cocci as a scarcely visible halo.

This micrococcus *stains* readily with the aniline colors; and also by Gram's method, which constitutes an important character for distinguishing it from Friedländer's bacillus.

Biological Characters.—Grows in the presence of oxygen—*aërobic*—but is also a *facultative anaërobic.* Like other micrococci, it has no spontaneous movements. It grows in a variety of culture media when they have a slightly alkaline reaction, but will not develop in a medium which contains the slightest trace of free acid. Nor will it grow at the ordinary room temperature. Scanty development may occur at a temperature of 22° to 24° C., but a temperature of 35° to 37° C. is most favorable for its growth, which is very rapid in a suitable liquid medium. In an infusion made from the flesh of a chicken or a rabbit it multiplies, in the incubating oven, with remarkable rapidity; at the end of six to twelve hours after inoculation the previously transparent fluid will be found to present a slight cloudiness and to be filled throughout with the cocci in pairs and short chains. It does not produce a milky opacity in liquid media, like the pus cocci, for example, but the fluid becomes slightly clouded; multiplication ceases at the end of about forty-eight hours or less, and the liquid medium again becomes transparent as a result of the subsidence of the cocci to the bottom of the receptacle.

It may be cultivated in flesh-peptone-gelatin, containing fifteen per cent of gelatin, at a temperature of 24° C., or in liquefied gelatin (ten per cent) in the incubating oven. In gelatin (fifteen per cent) stab cultures small white colonies develop all along the line of puncture, and in gelatin plates small, spherical, slightly granular, whitish colonies are formed: the *gelatin is not liquefied.* In agar plates extremely minute colonies are developed in the course of forty-eight hours, which resemble little, transparent drops of fluid, and under the microscope some of these are observed to have a compact, finely granular central portion surrounded by a paler, transparent, finely granular marginal zone. Upon the surface of nutrient agar or coagulated blood serum development occurs in the form of minute, transparent, jelly-like drops, which form a thin layer along the line of inoculation in "streak cultures"; and in agar stick cultures the growth along the line of puncture is

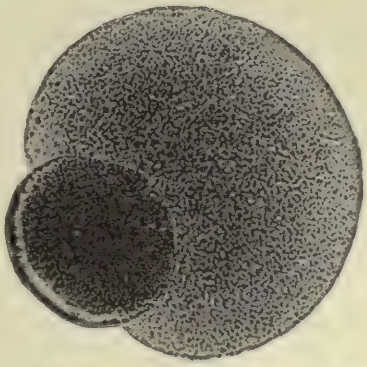

FIG. 94.—Single colony of Micrococcus pneumoniæ crouposæ upon agar plate, twenty-four hours old. × 100. (Fränkel and Pfeiffer.)

rather scanty, almost homogeneous, and semi-transparent. Upon potato no development occurs, even in the incubating oven. Milk is a favorable culture medium, and the casein is coagulated as a result of its presence.

It ceases to grow on solid media at about 40° C., and in favorable liquid media at 42° C. Its thermal death-point, as determined by the writer, is 52° C., the time of exposure being ten minutes. It loses its vitality in cultures in a comparatively short time—four or five days on agar—and is very sensitive to the action of germicidal agents. Its pathogenic power also undergoes attenuation very quickly when it is cultivated in artificial media, but may be restored by passing it through the bodies of susceptible animals. Attenuation of virulence may also be effected by exposing bouillon cultures to a temperature of 42° C. for twenty-four hours, or by five days' exposure to a temperature of 41° C.

Emmerich reported in 1891 to the Congress of Hygiene and Demography in London the results of experiments made by him relating to immunity in rabbits and mice. Rabbits were rendered immune by the intravenous injection of a very much diluted but virulent culture of the micrococcus. The flesh of these immune rabbits was rubbed up into a fine paste, and the juices obtained by compressing it in a clean, sterilized cloth. This bloody juice was kept for twelve hours at a temperature of 10° C., and then sterilized by passing it through a Pasteur filter. Some of this juice was injected into a rabbit, which with twenty-five others was then made to respire an atmosphere charged with a spray of a bouillon culture of the micrococcus. As a result of this all of the rabbits died except the one which had previously been injected with the immunizing juice. In a similar experiment upon mice six of these animals, which had previously been injected with the immunizing juice, survived the injection of a full dose of a virulent culture, while a control mouse, not previously injected with the juice, promptly died after receiving the same quantity of the virulent culture.

The writer in 1881, in experiments made to determine the value of various disinfectants, as tested upon this micrococcus, obtained experimental evidence that its virulence is attenuated by the action of certain antiseptic agents. Commenting upon the results of these experiments in my chapter on "Attenuation of Virus," in "Bacteria" (1884), I say :

> "Sodium hyposulphite and alcohol were the chemical reagents which produced the result noted in these experiments ; but it seems probable that a variety of antiseptic substances will be found to be equally effective when used in the proper proportion. Subsequent experiments have shown that neither of these agents is capable of destroying the vitality of this septic micrococcus in the proportion used (one per cent of sodium hyposulphite or

one part of ninety-five-per-cent alcohol to three parts of virus), and that both have a restraining influence upon the development of this microörganism in culture fluids."

The following results were obtained by the writer in his experiments (1881 and 1883) to determine the germicidal and antiseptic value of the agents named, as tested upon this micrococcus.

Alcohol.—A twenty-four-per-cent solution was effective upon bouillon cultures in two hours.

Boric Acid.—A saturated solution failed to destroy vitality after two hours' exposure, but 1 : 400 restrained development.

Carbolic Acid.—A one-per-cent solution destroys vitality in two hours, and 1 : 500 restrains development.

Cupric Sulphate destroys the virulence of the coccus in the blood of a rabbit in the proportion of 1 : 400 in half an hour.

Ferric Sulphate failed to destroy vitality in a saturated solution, but restrained development in the proportion of 1 : 200.

Hydrochloric Acid destroys the virulence of the blood of a rabbit containing this micrococcus in the proportion of 1 : 200.

Iodine, in aqueous solution with potassium iodide, destroys vitality in the proportion of 1: 1,000 and prevents development in 1: 4,000.

Mercuric Chloride.—One part in forty thousand prevents the development of this micrococcus, and 1 : 20,000 was found to destroy vitality in two hours.

Nitric Acid.—One part in four hundred destroyed the virulence of rabbit's blood containing this micrococcus.

Caustic Potash.—A two-per-cent solution destroyed vitality in two hours.

Potassium Permanganate.—A two-per-cent solution destroyed the virulence of rabbit's blood containing this coccus.

Salicylic Acid, dissolved by the addition of sodium biborate.—A solution of 1 : 400 prevented development.

Sulphuric Acid.—One part in two hundred destroys vitality, and 1 : 800 prevents development.

In a paper by Bordoni-Uffreduzzi relating to the resisting power of pneumonic virus for desiccation and light, the following results are given: Pneumonic sputum attached to cloths, when dried in the air and exposed to diffuse daylight, retained its virulence, as shown by injection in rabbits, for a period of nineteen days in one series of experiments and for fifty-five days in another. Exposed to direct sunlight the same material retained its virulence after twelve hours' exposure. Cultures have far less resistance, and the protection afforded by the dried albuminous material in which the micrococci were embedded, in the experiments referred to, probably accounts for the virulence being retained so long a time.

Kruse and Pansini (1892) have published an elaborate paper giving an account of their researches relating to "diplococcus pneumoniæ" and allied streptococci. We give below a summary statement of their results:

Many varieties were obtained by the observers named in their cultures from various sources—from the lungs of individuals dead from pneumonia, from pleuritic exudate, from pneumonic sputa, from bronchitic sputa, from the saliva of healthy persons, from the secretion in a case of subacute nasal catarrh, from the urine of a patient with nephritis.

Pure cultures were obtained by the use of agar plates or by inoculations into rabbits. In all about thirty varieties were obtained and cultivated through many successive generations. As a rule, the different varieties, which at first were seen to have the form of diplococci, when cultivated for a length of time in artificial media presented the form of streptococci ; and the elements which at first were lancet-shaped showed a tendency to become spherical.

The more virulent varieties usually presented the form of diplococci with lancet-shaped elements, or of short chains. A variety which formed long chains could be pronounced, in advance of the experiments on animals, to possess comparatively little virulence. When by inoculations in animals the virulence of such a variety was restored, the tendency to form chains was less pronounced.

Although, as a rule, no development occurs at 20° C., certain varieties were obtained which, after long cultivation in artificial media, showed a decided growth at 18° C.

Decided differences were shown by the cultures from various sources as regards their growth in milk. Out of eighty-four cultures from various sources eleven did not produce coagulation. As a rule, cultures which caused coagulation of milk were virulent for rabbits, and when such cultures lost their virulence they usually lost at the same time the power of coagulating milk. Virulent cultures die out sooner than those which have become attenuated by continuous cultivation in artificial media; the first, on the surface of agar, usually fail to grow at the end of a week, while the attenuated cultures may survive for three weeks or more.

Pathogenesis.—This micrococcus is very pathogenic for mice and for rabbits, less so for guinea-pigs. The injection of a minute quantity—0.2 cubic centimetre or less—of a virulent culture beneath the skin of a rabbit or a mouse usually results in the death of the animal in from twenty-four to forty-eight hours. The following is from the writer's first published paper (1881), and refers to the pathological appearances in rabbits :

"The course of the disease and the post-mortem appearances indicate that it is a form of septicæmia. Immediately after the injection there is a rise of temperature, which in a few hours may reach 2° to 3° C. (3.6° to 5.4° F.); the temperature subsequently falls, and shortly before death is often several degrees below the normal. There is loss of appetite and marked debility after twenty-four hours, and the animal commonly dies during the second night or early in the morning of the second day after the injection. Death occurs still more quickly when the blood from a rabbit recently dead is injected. Not infrequently convulsions immediately precede death.

"The most marked pathological appearance is a diffuse inflammatory œdema or cellulitis, extending in all directions from the point of injection, but especially to the dependent portions of the body. Occasionally there is a little pus near the puncture, but usually death occurs before the cellulitis reaches the point of producing pus. The subcutaneous connective tissue contains a quantity of bloody serum, which possesses virulent properties and which contains a multitude of micrococci. There is usually more or less inflammatory adhesion of the integument to the subjacent tissues. The liver is sometimes dark-colored and gorged with blood, but more frequently it is of a lighter color than normal and contains much fat. The spleen is either normal in appearance or enlarged and dark-colored. Changes in this organ are more marked in those cases which are of the longest duration.

"The blood commonly contains an immense number of micrococci, usually joined in pairs and having a diameter of about 0.5 μ. These are found in blood drawn from superficial veins, from arteries, and from the cavities of the heart immediately after death, and in a few cases their presence has been verified during life. Observations thus far made, however, indicate that it is only during the last hours of life that these parasites multiply in the circulating fluid, and in a certain proportion of the cases a careful search has failed to reveal their presence in the blood in post-mortem examinations made immediately after the death of the animal."

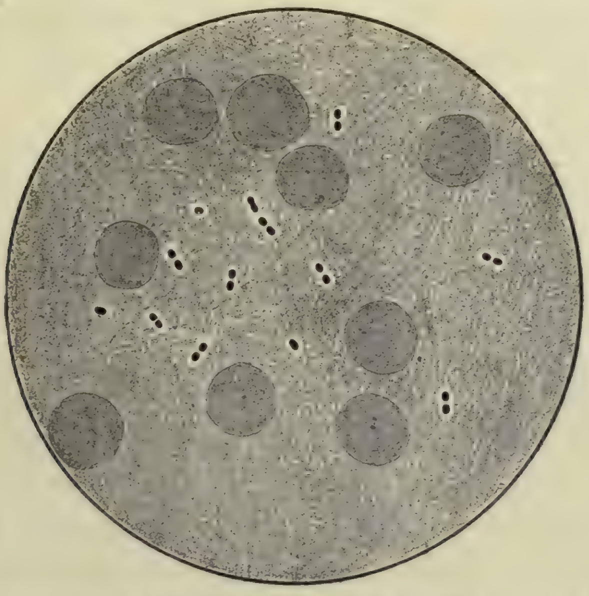

FIG. 95.—Micrococcus pneumoniæ crouposæ in blood of rabbit inoculated with pneumonic sputum. × 1,000.

In animals which are not examined until some hours after death a considerable increase in the number of micrococci occurs post mortem. The fact that this micrococcus varies very much as to its pathogenic power, as a result of conditions relating to the medium in which it develops, was insisted upon in my first published paper, and has been fully established by later researches (Fränkel, Gameléia). Susceptible animals inoculated with attenuated cultures acquire an immunity against virulent cultures.

In dogs subcutaneous injections usually give a negative result, or at most a small abscess forms at the point of inoculation. In a single experiment, however, the writer has seen a fatal result in a dog from the injection of one cubic centimetre of bloody serum from the subcutaneous connective tissue of a rabbit recently dead. This shows the intense virulence of the micrococcus when cultivated in the body of this animal. Pneumonia never results from subcutaneous injections into susceptible animals, but injections made through the thoracic walls into the substance of the lung may induce a typical fibrinous pneumonia. This was first demonstrated by Talamon (1883), who injected the fibrinous exudate of croupous pneumonia, obtained after death, or drawn during life by means of a Pravaz syringe from the hepatized portions of the lung, into the lungs of rabbits. According to Sée, eight out of twenty animals experimented upon exhibited "a veritable lobar, fibrinous pneumonia, with pleurisy and pericarditis of the same nature." Gameléia has also induced pneumonia in a large number of rabbits, and also in the dog and the sheep, by injections directly into the pulmonary tissue. Sheep were found to survive subcutaneous inoculations, unless very large doses (five cubic centimetres) of the most potent virus were injected. But intrapulmonary inoculations invariably induced a typical fibrinous pneumonia which usually proved fatal. In dogs similar injections gave rise to a "frank, fibrinous pneumonia which rarely proved fatal, recovery usually occurring in from ten to fifteen days, after the animal had passed through the stages of red and gray hepatization characteristic of this affection in man."

Monti claims to have produced typical pneumonia in rabbits by injecting cultures of this micrococcus into the trachea.

From the evidence obtained in these experimental inoculations, and that recorded relating to the presence of this micrococcus in the fibrinous exudate of croupous pneumonia, we are justified in concluding that it is the usual cause of this disease, and consequently have described it under the name Micrococcous pneumoniæ crouposæ. We prefer this to the name commonly employed by German authors—"diplococcus pneumoniæ"—because this micrococcus, although commonly seen in pairs, forms numerous short chains of three or four elements in cultures in liquid media, and upon the surface of nutrient agar may grow out into long chains. It would, therefore, more properly be called a streptococcus than a diplococcus.

While the micrococcus of pneumonia is not usually seen in the blood in cases of pneumonia it is probably present in small numbers, and secondary infection of the kidneys appears to be a common occurrence. Thus Fränkel and Reiche (1894) report that in twenty-two cases out of twenty-four in which they had an opportunity to examine the kidneys, this micrococcus was present. It was found especially in the larger branches of the veins and arteries, but also in the intertubular vessels and the glomeruli. The kidneys gave evidence of degenerative changes, and it is probable that the "pneumococcus" would have been found in the urine of some of these cases if a bacteriological examination had been made during life.

VII.

PATHOGENIC MICROCOCCI NOT DESCRIBED IN SECTIONS V. AND VI.

DIPLOCOCCUS INTRACELLULARIS MENINGITIDIS.

DISCOVERED by Weichselbaum (1887) in the exudate of cerebrospinal meningitis (six cases), for the most part within the cells.

Morphology.—Micrococci, usually united in pairs, in groups of four, or in little masses; sometimes solitary and larger (probably being upon the point of dividing). Distinguished by their presence in the interior of pus cells in the exudate, in this respect resembling the gonococcus.

Stain best with Löffler's alkaline solution of methylene blue. Do not retain their color when treated with iodine solution (Gram's method).

Biological Characters.—This micrococcus does not grow at the room temperature, but upon nutrient agar an abundant development occurs in the incubating oven. Upon the surface of agar a tolerably luxuriant, viscid growth, which by reflected light is gray and by transmitted light grayish-white; along the line of puncture growth occurs only near the surface, indicating that this micrococcus will not grow in the absence of oxygen. Upon plates made from agar-agar (one per cent) and gelatin (two per cent) very small colonies are formed in the interior of the mass, and larger ones, of a grayish color, on the surface. The former, under the microscope, are seen to be round or slightly irregular, finely granular, and of a yellowish-brown color. The superficial colonies have a yellowish-brown nucleus, surrounded by a more transparent zone. The growth upon coagulated blood serum is very scanty, as is that in bouillon; no growth occurs upon potato. This micrococcus quickly loses its power of reproduction in artificial cultures—within six days—and should be transplanted to fresh material at short intervals—two days.

Pathogenesis.—Mice are especially susceptible, and usually die within forty-eight hours after inoculation. Also pathogenic for guinea-pigs, rabbits, and dogs.

MICROCOCCUS TETRAGENUS.

First described by Gaffky (Flügge). Obtained by Koch and Gaffky (1881) from a cavity in the lung in a case of pulmonary phthisis. Since found occasionally in normal saliva (three times in fifty persons examined by Biondi), and in the pus of acute abscesses (Steinhaus, Park, Vangel). Rather common in the sputum of phthisical cases.

Morphology.—Micrococci, having a diameter of about one μ, which divide in two directions, forming tetrads, which are enclosed in a transparent, jelly-like envelope—especially well developed as seen in the blood and tissues of inoculated animals. In cultures the cocci are seen in the various stages of division, as large single cells, pairs of oval elements, or groups of four resulting from the transverse division of these latter.

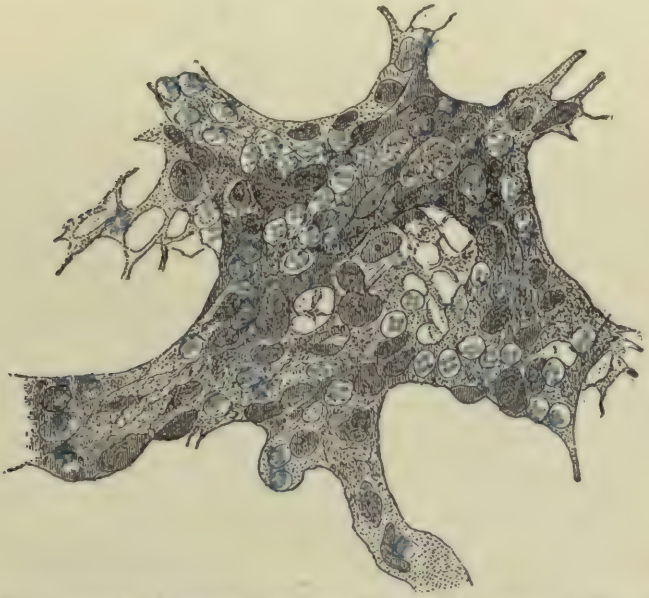

FIG. 96.—Micrococcus tetragenus; section of lung of mouse. × 800. (Flügge.)

Stains quickly with aniline colors, and in preparations from the blood of an inoculated animal the transparent envelope may also be feebly stained. Stains also by Gram's method.

Biological Characters.—This micrococcus grows, rather slowly, in nutrient gelatin at the ordinary room temperature, without liquefaction of the gelatin. Upon gelatin plates small white colonies are developed in from twenty-four to forty-eight hours, which under the microscope, with a low power, are seen to be spherical or lemon-shaped, finely granular, and with a mulberry-like surface. When they come to the surface they form white, elevated, and rather thick masses having a diameter of one to two millimetres. In gelatin stab cultures a broad and thick white mass forms upon the surface, and along the line of puncture a series of round, milk-white or yellowish masses form, which usually remain distinct, but may become confluent. Upon the surface of agar the growth is similar to that upon gelatin, or in streak inoculations may consist of a series of spherical, white colonies. Upon cooked potato a thick, viscous layer is formed of milk-white color ; the growth upon blood serum is also abundant, especially in the incubating oven. This micrococcus is a facultative anaërobic.

Pathogenesis.—Subcutaneous inoculation of a culture of this micrococcus in minute quantity is fatal to white mice in from two to six days. The animals remain apparently well for the first day or two, then remain quiet and somnolent until death occurs. The cocci are found in comparatively small numbers in the blood of the heart, but are more numerous in the spleen, lungs, liver, and kidneys, from which organs beautiful stained preparations may be made showing the tetrads surrounded by their transparent capsule. Common house mice and field mice are, for the most part, immune, as are the rabbit and the dog. Guinea-pigs sometimes die from general infection, and sometimes a local abscess is the only result of a subcutaneous inoculation.

MICROCOCCUS BOTRYOGENUS (Rabe).

Synonyms.—Micrococcus of "myko-desmoids" of the horse; Micrococcus askoformans (Johne) ; Ascococcus Johnei (Cohn).

First described by Bollinger (1870) ; morphological characters and location in the diseased tissues described by Johne (1884) ; biological characters determined by Rabe (1886).

Is found in certain diffused or circumscribed growths in the connective tissue of horses—"myko-desmoids."

Morphology.—Micrococci, having a diameter of 1 to 1.5 μ, usually united in pairs.

In the tissues the cocci are united in colonies of fifty to one hundred μ in diameter, and these are associated in mulberry-like masses visible to the naked eye. The separate colonies are enclosed in a homogeneous, transparent envelope—as in Ascococcus Billrothii. This is not the case, however, in cultures in artificial media.

Stains with the aniline colors.

Biological Characters.—In gelatin plate cultures spherical, sharply defined, silver-gray colonies are developed ; later these have a yellowish color and a metallic lustre, and the plate presents the appearance of being powdered with grains of pollen. It gives off a peculiar fruit-like odor, reminding one of the odor of strawberries. In gelatin stab cultures growth occurs along the line of puncture as a pale grayish-white line, which later becomes milk-white ; an air bubble forms near the surface of the gelatin ; very slight liquefaction occurs in the immediate vicinity of the line of growth, and after a time the grayish-white thread sinks into an irregular mass, lying at the bottom of the puncture. Upon nutrient agar scarcely any development occurs. Upon potato the growth is abundant, in the form of a pale-yellow, circular layer, and the culture gives off the peculiar odor above described.

Pathogenesis.—When inoculated into guinea-pigs general infection and death result. In sheep and goats it produces a local inflammatory œdema and sometimes necrosis of the tissues. In horses inoculated subcutaneously an inflammatory œdema first occurs, followed at the end of from four to six weeks by the development of new growths in the connective tissue, resembling the tumors found in cases of the disease in the animal from which the micrococcus in question was first cultivated. These tumors contain characteristic mulberry-like conglomerations of colonies made up of the coccus.

MICROCOCCUS OF MANFREDI.

Synonym.—Micrococcus of progressive granuloma formation.

Obtained by Manfredi (1886) from the sputum of two cases of croupous pneumonia following measles.

Morphology.—Oval micrococci, having a diameter of 0.6 to 1.0 μ and from 1.0 to 1.5 μ in length ; usually associated in pairs, and occasionally in short chains containing three or four elements.

Stains with the aniline colors and by Gram's method.

Biological Characters.—Aërobic ; does not liquefy gelatin. Upon gelatin plates forms small, spherical colonies, at first grayish-white, which spread out upon the surface as thin, transparent plates, which by transmitted light have a bluish, by reflected light a pearl-gray color. Later these become thicker and have a pearly lustre. Under the microscope (forty to fifty diameters) the colonies are seen to be slightly granular and the margins have an irregular outline. In gelatin stab cultures a scanty growth occurs along the line of puncture, and a rather thin and limited growth about the point of inoculation. Upon blood serum a thin, greenish-yellow layer, which has irregular margins and a slightly granular, shining surface, is developed. The growth upon potato, at 37° C., is scanty, and consists of a very thin, moist layer, which has a yellowish color and is slightly granular. Growth occurs in favorable media—bouillon, gelatin—at temperatures of 18° to 48° C., but ceases at a temperature of 48° to 50° C.

Pathogenesis.—Pathogenic for dogs, rabbits, guinea-pigs, mice, and birds. In mammals the principal pathological appearance resulting from infection consists in the formation of " granulation tumors" in the parenchymatous organs. These vary in size from that of a millet seed to that of a pea, and undergo caseation. They contain the micrococcus and are infectious. Mammals die in from nine to fifteen days; birds in from one to three or four, and without the formation of the characteristic granuloma, but with general infection of the blood. Cultures which have been kept for several months retain their pathogenic power.

MICROCOCCUS OF BOVINE MASTITIS (Kitt).

Obtained by Kitt (1885) from the udder of cows suffering from mastitis and giving milk mixed with pus.

Morphology.—Micrococci, having a diameter of 0.2 to 0.5 μ, solitary, united in pairs, in irregular groups, and occasionally in chains.

Stains with the aniline colors.

Biological Characters.—Does not liquefy gelatin. Upon gelatin plates forms spherical, translucent, glistening colonies, the size of a hemp seed to that of a pin's head; in gelatin stab cultures a nail-shaped growth occurs, the mass at the point of puncture being opaque and of a white color. Upon potato, colonies are quickly developed which have a grayish-white or dirty yellow color, and after a few days have a shining, wax-like appearance. Grows rapidly in milk, causing an acid reaction; in six hours in the incubating oven the milk is pervaded by the micrococcus, or in twelve hours at 20° C.

Pathogenesis.—Injection of pure cultures, suspended in distilled water, into the mammary glands of cows, produces typical, acute, purulent mastitis (Kitt). The micrococcus produced the same result after having been cultivated in artificial media for a year. Subcutaneous inoculations in cows, pigs, guinea-pigs, rabbits, and mice were without result. Injections into the mammary gland of goats were also without effect.

MICROCOCCUS OF BOVINE PNEUMONIA (?).

Isolated by Poels and Nolen (1886) from the lungs of cattle suffering from "Lungenseuche" (infectious pleuro-pneumonia of cattle).

Morphology.—Micrococci, varying considerably in size—average diameter 0.9 μ; solitary, in pairs, or in chains containing several elements; surrounded by a transparent capsule, which stains with difficulty.

Stains with all the aniline colors, and with difficulty by Gram's method.

Biological Characters.—Does not liquefy gelatin, and grows like the bacillus of Friedländer in gelatin stab cultures (nail-shaped growth). In gelatin plates the colonies are spherical, white, and have a very faint yellowish tinge. Grows more rapidly on agar in the incubating oven, and upon potato in the form of a very pale-yellowish layer. Is destroyed by a temperature of 66° C. maintained for fifteen minutes.

Pathogenesis.—Pure cultures injected into the lungs of dogs, rabbits, and guinea-pigs are said to give rise to pneumonic inflammation, and similar results were obtained by injection into the trachea of dogs and by inhalation experiments. Injection of a pure culture into the lungs of a cow caused extensive pneumonic changes; but these did not entirely correspond with the appearances found in the lungs of cattle suffering from infectious pneumonia. Cattle inoculated with a pure culture, by means of a sterilized lancet, did not fall sick, but are believed by Poels and Nolen to have been protected from the disease by such inoculations.

The specific relation of the micrococcus above described to the disease with which it was associated, in the researches of the authors mentioned, has not been established by subsequent investigations.

STREPTOCOCCUS SEPTICUS (Flügge).

Found by Nicolaier and by Guarneri in unclean soil during researches made in Flügge's laboratory in Göttingen.

Morphology.—Cannot be distinguished from Streptococcus pyogenes, but does not so constantly form chains, being found in the tissues of inoculated animals, for the most part in pairs.

Biological Characters.—Grows more slowly than Streptococcus pyogenes; in gelatin plates very minute colonies first appear at the end of three or four days, or along the line of puncture in gelatin stick cultures after five or six days. Does not liquefy gelatin.

Pathogenesis.—Is very pathogenic for mice and for rabbits, causing death from general infection in two or three days.

STREPTOCOCCUS BOMBYCIS.

Synonym.—Microzyma bombycis (Béchamp).

Found in the bodies of infected silkworms suffering from *la flacherie* (maladie des morts-plats). Etiological relation established by Pasteur.

Morphology.—Oval cells, not exceeding 1.5 μ in diameter, in pairs or in chains.

Biological Characters.—Not determined with precision.

Pathogenesis.—The infected silkworm ceases to eat, becomes weak, and dies. Its body is soft and diffluent, and at the end of twenty-four to forty-eight hours is filled with a dark-brown fluid and with gas.

NOSEMA BOMBYCIS.

Synonyms.—Micrococcus ovatus; Panhistophyton ovatum.

Found in the blood and all of the organs of silkworms infected with pébrine (Fleckenkrankheit).

First observed by Cornalia. Etiological relation established by Pasteur.

Morphology.—Shining, oval cells, three to four μ long and two μ broad; solitary, in pairs, or in irregular groups.

Biological Characters.—Not determined with precision.

Pathogenesis.—Dark spots appear upon the skin of infected silkworms, which lose their appetite, become slender and feeble, and soon die. The oval corpuscles are found in all of the organs, and also in the eggs of butterflies hatched from infected larvæ. Some authors are of the opinion that the oval corpuscles found in this disease do not belong to the bacteria, but to an entirely different class of microörganisms—the Psorospermia (Metschnikoff).

MICROCOCCUS OF HEYDENREICH.

Synonyms.—Micrococcus of Biskra button—*Fr.* "clou de Biskra"; *Ger.* "Pendesche Geschwur."

Found by Heydenreich (1888) in pus and serous fluid obtained from the tumors and ulcers in the Oriental skin affection known as Biskra button.

Morphology.—Diplococci, from 0.86 to 1 μ in length, surrounded by a capsule; sometimes associated to form tetrads.

Stains with the usual aniline colors.

Biological Characters.—An *aërobic*, *liquefying* micrococcus. Grows in the usual culture media at the room temperature. In *gelatin stick cultures*, at 20° C., at the end of forty-eight hours growth occurs along the line of puncture in the form of small, crowded colonies, which produce a grayish-white line; upon the surface a thin, circular layer of a yellowish-white color is developed. At the end of three to four days liquefaction commences near the surface, where a funnel is formed which extends until about the fourteenth day, when the gelatin is completely liquefied. Upon the surface of agar, at 37° C., a grayish-white or yellowish layer is formed at the end of twenty-four hours, which has a varnish-like lustre. Upon *potato*, at 30° to 35° C., at the end of forty-eight hours a white or yellow layer has developed.

Pathogenesis.—According to Heydenreich, inoculations in rabbits, dogs, chickens, horses, and sheep cause a skin affection which is identical with that which characterizes Biskra button in man. When rubbed into the healthy skin of man it also produces the development of abscesses.

MICROCOCCUS ENDOCARDITIDIS RUGATUS (Weichselbaum).

Obtained by Weichselbaum (1890) from the affected cardiac valves in a fatal case of ulcerative endocarditis.

Morphology.—Micrococci, resembling the staphylococci of pus in dimensions and mode of grouping; solitary, in pairs, in groups of four, or in irregular masses.

Biological Characters.—An *aërobic* micrococcus. Does not grow at the room temperature. Upon *agar plates*, at 37° C., at the end of three or four days the superficial colonies consist of a small, brown, central mass surrounded by a granular, semi-transparent, grayish marginal zone; gradually they attain a characteristic wrinkled appearance; the deep colonies, under a low power, are irregular, finely granular, and contain a large central, yellowish-brown nucleus surrounded by a narrow, grayish-brown peripheral zone. In *agar stab cultures* small, spherical colonies are formed upon the surface, which become confluent, forming a grayish-white, wrinkled layer which has a stearin-like lustre and is very viscid; a scanty growth occurs along the line of puncture. Upon *potato*, at 37° C., a scanty development occurs in the form of a small, dry, pale-brown mass. Upon blood serum isolated or confluent, colorless colonies are formed the size of a poppy seed; these are closely adherent to the surface of the culture medium.

Pathogenesis.—When injected subcutaneously into the ear of a rabbit it produces tumefaction and redness; in guinea-pigs, formation of pus. When injected into the circulation of dogs, after injury to the aortic valves, an endocarditis is developed.

MICROCOCCUS OF GANGRENOUS MASTITIS IN SHEEP.

Obtained by Nocard (1887) from the milk of sheep suffering from gangrenous mastitis (mal de pis or d'araignée), a fatal disease which attacks especially sheep which are being milked for the manufacture of cheese, at Roquefort and elsewhere in France.

Morphology.—Micrococci, solitary, in pairs, or in irregular groups, resembling the staphylococci of pus in dimensions and arrangement.

Stains with the usual aniline colors and also by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic, liquefying* micrococcus. Grows at the room temperature in the usual culture media. Upon *gelatin plates*, at the end of forty-eight hours, the colonies are spherical and white in color; under a low power the superficial colonies are circular in outline, homogeneous, and brown in color; they are surrounded by a semi-transparent aureole; liquefaction around the superficial colonies occurs sooner than around those beneath the surface of the gelatin. In gelatin stick cultures, at 18° to 20° C., on the second day liquefaction of the gelatin commences near the surface; by the fifth day a pouch of liquefied gelatin has formed, which has the shape of an inverted cone; at the bottom of this an abundant deposit of micrococci is seen, while the liquefied gelatin above is clouded throughout. In *agar stick cultures* development occurs upon the surface as a thick white layer, which gradually extends over the entire surface, and after a time acquires a yellowish tint; development also occurs along the line of puncture. Upon *potato* a thin, viscid, grayish layer is slowly developed; the outline is irregular and the edges thicker than the central portion; the central portion of this layer gradually acquires a yellow color, while the periphery remains of a dirty-white or grayish color. *Blood serum* is liquefied by this micrococcus.

Pathogenesis.—A few drops of a pure culture injected subcutaneously or into the mammary gland of sheep cause an extensive inflammatory œdema and the death of the animal in from twenty-four to forty-eight hours. A cubic centimetre injected into the mammary gland of a goat produced no result; the horse, the calf, the pig, the cat, chickens, and guinea-pigs also proved to be immune. Subcutaneous injections in rabbits produce an extensive abscess at the point of inoculation.

STREPTOCOCCUS OF MASTITIS IN COWS.

Obtained by Nocard and Mollereau (1887) from the milk of cows suffering from a form of chronic mastitis (mammite contagieuse).

Morphology.—Spherical or oval cocci, a little less than one μ in diameter, usually united in long chains.

Stains with the usual aniline colors and also by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying* streptococcus. Grows in the usual culture media at the room temperature. Develops rapidly in milk or in bouillon at a temperature of 16° to 30° C. The milk of a cow suffering from the form of mastitis produced by this micrococcus, when drawn with proper precautions in sterilized test tubes, at the end of twenty-four hours is acid in reaction; the lower two-thirds of the tube is filled with an opaque, dirty-white, homogeneous deposit, and above this is an opalescent, serous fluid of a bluish or dirty-yellow or slightly reddish color, according to the age of the lesion. A drop of this milk examined under the microscope shows the presence of the streptococcus in great numbers. The addition of two to five per cent of glucose or of glycerin to bouillon makes it a more favorable culture medium; the reaction should be neutral or slightly alkaline, as this streptococcus does not grow readily in an acid medium, although it produces an acid reaction in media containing sugar, the acid formed being lactic. In *gelatin stab cultures* the growth upon the surface is scanty, in the form of a thin pellicle around the point of puncture; along the line of inoculation minute, opaque, granular colonies are developed, which, being closely crowded, form a thick line with jagged margins.

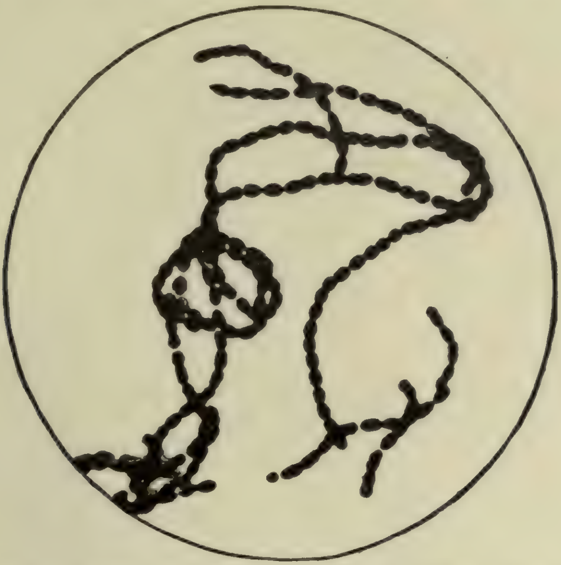

FIG. 97.—Streptococcus of mastitis in cows (Nocard).

In *agar stab cultures* the growth is similar but more abundant. Upon the surface of nutrient gelatin, agar, or blood serum a large number of minute, spherical, semi-transparent colonies are developed among the impfstrich; these have a bluish tint by reflected light; they may become confluent, forming a thin layer with well-defined margins. Upon *gelatin plates*, at 16° to 18° C., colonies are first visible at the end of two or three days; they are spherical and slightly granular, at first transparent and later of a pale-yellow color by transmitted light, which gradually becomes brown. At the end of five or six weeks the colonies are still quite small, well defined, and opaque.

Pathogenesis.—Pure cultures injected into the mammary gland of cows and goats gave rise to a mastitis resembling in its development that from which the streptococcus was obtained in the first instance. Injections into the cavity of the abdomen or into a vein, of one cubic centimetre of a pure culture, gave a negative result in dogs, cats, rabbits, and guinea-pigs.

DIPLOCOCCUS OF PNEUMONIA IN HORSES.

Obtained by Schütz (1887) from the lungs of horses affected with pneumonia.

Morphology.—Oval cocci, usually in pairs, surrounded by a homogeneous, transparent capsule.

Does not stain by Gram's method.

Biological Characters.—An *aërobic*, *non-liquefying* micrococcus. Grows at the room temperature. Upon *gelatin plates* forms small, spherical, white colonies.

In *gelatin stick cultures* grows along the line of puncture as small, white, separate colonies, which grow larger without becoming confluent. Upon the surface of *agar* small transparent drops are developed along the impfstrich.

Pathogenesis.—The injection of a pure culture into the lung of a horse produces pneumonia and causes its death in eight or nine days. Pathogenic for rabbits, guinea-pigs, and mice.

STREPTOCOCCUS CORYZÆ CONTAGIOSÆ EQUORUM.

Obtained by Schütz (1888) from pus from the lymphatic glands involved in horses suffering from the disease known in Germany as Druse des Pferdes.

Morphology.—Oval cocci, in pairs, in chains containing three or four elements, or in long chaplets.

Stains with the usual aniline colors—very intensely with Weigert's or Ehrlich's solution.

Biological Characters.—An *aërobic* and *facultative anaërobic* micrococcus. Grows slowly at the room temperature, more rapidly at 37° C. Upon *gelatin plates* at the end of three to five days minute colonies become visible; these never exceed the size of a pin's head. In *gelatin stab cultures* growth upon the surface is scanty or absent; along the line of puncture minute colonies are developed in rows. Upon *agar plates*, at 37° C., at the end of twenty four hours lentil-shaped colonies are developed the size of a pin's head; under a low power the superficial colonies are seen to have a well-defined, opaque nucleus surrounded by a grayish, transparent marginal zone, which represents a half-fluid, slimy growth which does not extend after the third day and later disappears entirely; the deep colonies are at first well-defined, and later surrounded by wing-like outgrowths. Upon *blood serum*, at 37° C., yellowish, transparent drops are first developed; these become confluent and form a viscid and tolerably thick layer; this later becomes dry and iridescent.

Pathogenesis.—Pathogenic for horses and for mice, producing in these animals an abscess at the point of inoculation, and metastatic abscesses in the neighboring lymphatic glands. Not pathogenic for rabbits, guinea-pigs, or pigeons.

HÆMATOCOCCUS BOVIS (Babes).

Obtained by Babes (1889) from the blood and various organs of cattle which had died of an epidemic malady (in Roumania) characterized by hæmoglobinuria. The cocci are found in the blood in great numbers, for the most part enclosed in the red corpuscles.

Morphology.—Biscuit-shaped cocci united in pairs; sometimes oblong in form, isolated or united in groups; the free cocci are surrounded by a pale-yellowish, shining aureole of 0.5 to 1 μ in diameter.

Stains best with Löffler's solution of methylene blue; does not stain by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying* micrococcus. Grows very slowly at the room temperature—not below 20° C. In the incubating oven grows in the usual culture media. In *gelatin stab cultures* a scanty development of small, white colonies occurs along the line of puncture. Upon the *surface of agar* small, transparent drops are developed along the impfstrich. Upon *potato*, at 37° C., a thin, broad, yellowish, shining layer is developed in the course of a few days—scarcely visible. Upon *blood serum* small, moist, transparent colonies are developed.

Pathogenesis.—Pathogenic for rabbits and rats, which die in from six to ten days after inoculation with a pure culture; the spleen is found to be enlarged, the lungs hyperæmic, and a bloody serum is found in the cavity of the abdomen; the cocci are present in the blood in considerable numbers, but are rarely seen in the red corpuscles. Inoculations in oxen, horses, goats, sheep, guinea-pigs, and birds were without effect.

STREPTOCOCCUS PERNICIOSUS PSITTACORUM.

Micrococcus of gray parrot disease. Eberth and Wolff have described an infectious disease of gray parrots, which is said to be extremely fatal among the imported birds. The disease is characterized by the formation of nodules upon the surface and in the interior of various organs, and especially in the liver. Micrococci of medium size are found in these nodules and in blood from the heart; these are sometimes in chains. Microscopic examination of stained sections shows that these cocci are directly related to the tissue necrosis which characterizes the disease. But the micrococcus has not been cultivated and its biological characters are undetermined.

STREPTOCOCCUS AGALACTIÆ CONTAGIOSÆ.

Obtained by Adametz (1894) from the milk of cows suffering from mastitis (Gelben Galt). According to Adametz all of the streptococci which have been described by different investigators (Kitt, Nocard and Mollereau, Guillebeau, and others) are probably varieties of a single species.

Morphology.—Spherical cocci in short chains—1 μ in diameter.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying* streptococcus.

Upon gelatin plates forms flat, transparent, white or bluish-white, slimy colonies, having a slight pearly lustre and an irregular outline. In nutrient gelatin containing five per cent of milk sugar the colonies, at the end of eight days, have a diameter of 0.85 to 1 millimetre; they are milk-white and of a semi-fluid, slimy consistence.

Upon agar plates the deep colonies are punctiform and white in color—under a low power they are seen to have an irregular dentate contour and a brownish color; the superficial colonies gradually assume the appearance of transparent, flat drops having a diameter of 0.5 to 0.7 millimetre. In sterilized milk fermentation occurs, at 37° C., in from twenty to twenty-four hours; some hours later the casein is precipitated, fine gas bubbles are seen in the lower part of the fluid and a foam upon the surface; the reaction is acid and the casein is not peptonized. The power of producing acid and gas is diminished or lost after a few successive cultures have been made.

Streptococcus mastitis sporadicæ (Guillebeau) is said by Adametz to be distinguished from the streptococcus above described (No. 44A) by being smaller—0.5 μ in diameter—and by the fact that the cultures do not lose the power of producing fermentation in milk.

MICROCOCCUS MELITENSIS.

Surgeon-Major Bruce, of the British army, in 1887 demonstrated the etiological relation of a micrococcus, now known as *Micrococcus melitensis*, to the infectious disease known as *Malta fever* (synonyms: Mediterranean fever; Neapolitan fever; Rock fever of Gibraltar, etc.). Subsequent researches show that this fever is not restricted to the Mediterranean region, and it will probably be found to have an extensive area of prevalence on both continents. Cases have been recognized in America and by medical officers of the army stationed in the Philippine Islands. Curry (Captain and Assistant Surgeon United States Volunteers), in a recent report to the Surgeon-General of the army, says:

> "I had the honor to report to the Surgeon-General of the Army on January 2d, 1900, four cases of Mediterranean or Malta fever, which came under my observation, while on duty as pathologist to the 1st Reserve Hospital in Manila, P. I., cases occurring among our troops and originating on the Island of Luzon.
>
> "Later, in a report on the 'Diseases of the Philippine Islands,' I reported twelve additional cases. In all these cases a positive serum reaction with the Micrococcus melitensis was obtained, and the clinical history of the cases corresponds with the descriptions of Malta fever as given by the English army surgeons Bruce, Hughes, Wright, Semple, and others, and that described by Manson. Included in these sixteen cases is one autopsy.
>
> "In my report on the 'Diseases of the Philippine Islands,' under the heading of 'Fevers of the Philippines,' I expressed the belief that 'Malta fever is *not* an *uncommon* disease in the Philippine Islands,' and that it appeared that 'Malta fever is by no means as limited geographically as has been thought heretofore.'
>
> "Our experience here in the Army and Navy General Hospital, Hot Springs, Ark., has convinced me that Malta fever is widespread in tropical and sub-tropical regions. We are but having a repetition of the experience of the English army surgeons at the Royal Victoria Hospital, Netley.
>
> "Among the soldiers and sailors, here in our wards, who have been returned from tropical stations, we have found already *four* to have Malta fever. These four cases came from widely separated stations. Two cases are in soldiers, one from the Philippines, and one from Cuba, and two are among sailors of the United States navy who were recently returned from South Atlantic stations.
>
> "All four cases entered this hospital with a diagnosis of rheumatism."

Morphology.—Micrococci, about 0.5 μ in diameter, usually solitary or in pairs; occasionally short chains are seen in cultures. In old cultures kept at the room temperature the cells may be oval or elongated.

Biological Characters.—An *aërobic*, *non-liquefying* micrococcus. Does not stain by Gram's method. Grows best in nutrient agar. In stab cultures no growth is seen for several days. "At length the growth appears as pearly-white spots scattered around the point of puncture and minute, round, white colonies are also seen along the course of the needle track"; these increase in size, and after some weeks a rosette-shaped growth is seen upon the surface, and the growth along the line of puncture has a yellowish-brown color. At the end of nine or ten days, at 37° C., some of the colonies on the surface of nutrient agar are as large as No. 4 shot; by transmitted light they have a yellowish color at the centre, and the periphery is bluish-white; by reflected light they have a milky-white color. At 25° C. colonies first become visible at the end of about seven days, at 37° C. in three to four days. Does not grow upon potato. Very scanty growth upon nutrient gelatin at 22° C. at the end of a month.

This micrococcus has usually been described as non-motile, but Gordon has demonstrated that it has from one to four flagella, which are difficult to demonstrate by the usual staining methods.

Pathogenesis.—Pathogenic for monkeys, which suffer from fever as a result of subcutaneous inoculations and usually die in from thirteen to twenty-one days. The spleen is found to be enlarged and contains the micrococcus. Not pathogenic for mice, guinea-pigs, or rabbits.

In man the micrococcus is found in large numbers in the spleen, which is greatly enlarged.

Widal Reaction.—The blood serum of patients suffering from Malta fever and of individuals who have recently recovered from the disease causes the agglutination of Micrococcus melitensis in recent cultures. According to Wright and Smith this reaction may be manifested a year after recovery. Dilution of 1:1000 will in exceptional cases give a distinct agglutinating effect.

VIII.

THE BACILLUS OF ANTHRAX.

[*Fr.*, CHARBON ; *Ger.*, MILZBRAND.]

ANTHRAX is a fatal infectious disease which prevails extensively among sheep and cattle in various parts of the world, causing heavy losses. In Siberia it constitutes a veritable scourge and is known there as the Siberian plague ; it also prevails to a considerable extent in portions of France, Hungary, Germany, Persia, and India, and local epidemics have occasionally occurred in England, where it is known under the name of splenic fever. It does not prevail in the United States. In infected districts the greatest losses are incurred during the summer season.

In man accidental inoculation may occur among those who come in contact with infected animals, and especially during the removal of the skin and cutting up of dead animals, when there is any cut or abrasion upon the hands. A malignant pustule is developed as the result of such inoculation, but, as a rule, general infection does not occur, as is the case when inoculations are made into the more susceptible lower animals—rabbit, guinea-pig, mouse. Those who handle the hair, hides, or wool of infected animals are also liable to contract the disease by inoculation through open wounds, or by the inhalation of dust containing spores of the anthrax bacillus. Cases of pulmonic anthrax, known formerly in England as "wool-sorters' disease," have been occasionally observed in England and in Germany, and are now recognized as being due to infection through the lungs in the manner indicated.

The French physician Davaine, who had observed the anthrax bacillus in the blood of infected animals in 1850, communicated to the French Academy of Sciences the results of his inoculation experiments in 1863 and 1864, and asserted the etiological relation of the bacillus to the disease with which his investigations showed it to be constantly associated. This conclusion was vigorously contested by conservative opponents, but has been fully established by subsequent investigations, which show that the bacillus, in pure cultures, induces anthrax in susceptible animals as certainly as does the blood of an animal recently dead from the disease.

Owing to the fact that this was the first pathogenic bacillus cultivated in artificial media, and to the facility with which it grows in various media, it has served more than any other microörganism for researches relating to a variety of questions in pathology, general biology, and public hygiene, some of which are discussed in other sections of this volume.

BACILLUS ANTHRACIS.

Synonyms.—Milzbrandbacillus, *Ger.;* Bactéridie du charbon, *Fr.*

First observed in the blood of infected animals by Pollender (1849) and by Davaine (1850). Etiological relation affirmed by Davaine (1863), and established by the inoculation of pure cultures by Pasteur (1879) and by many other investigators.

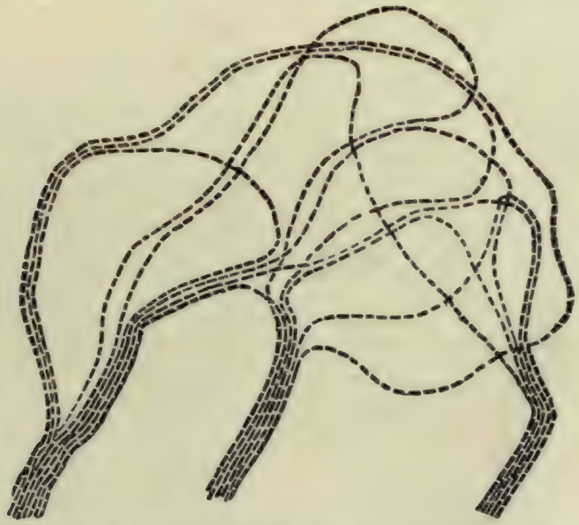

FIG. 98.—Bacillus anthracis, from a culture, showing development of long threads in convoluted bundles. × 300. (Klein.)

Morphology.—Rod-shaped bacteria having a breadth of 1 to 1.25 μ, and 5 to 20 μ in length; or, in suitable culture media, growing out into long, flexible filaments, which are frequently united in twisted, cord-like bundles. These filaments in hanging-drop cultures, before the development of spores, appear to be homogeneous; or the protoplasm is clouded and granular, but without distinct segmentation. But in stained preparations the filaments are seen to be made up of a series of rectangular, deeply stained segments. In hanging-drop cultures the ends of the rods appear rounded, but in stained preparations from the blood of an infected animal they are seen to present a slight concavity, and a lenticular interspace is formed where two rods come together. The diameter of the rods varies considerably in different culture media; and in old cultures irregular forms are frequently seen—"involution forms."

Under favorable conditions endogenous spores are developed in the long filaments which grow out in artificial culture media. These first appear as refractive granules distributed at regular intervals in the segments of the protoplasm, which gradually disappear as the spores are developed; and these are left as oval, highly refractive bodies, held together in a linear series by the cellular envelope, and subsequently set free by its dissolution. The germination of these reproductive bodies results in the formation of rods and spore-bearing filaments like those heretofore described. In this process the spore is first observed to lose its brilliancy, from the absorption of moisture, a prominence occurs at one end of the oval body, and soon the external envelope — exosporium—is ruptured, permitting the softened protoplasmic contents enclosed in the internal spore membrane—endosporium—to escape as a short rod, to which the empty exosporium sometimes remains attached.

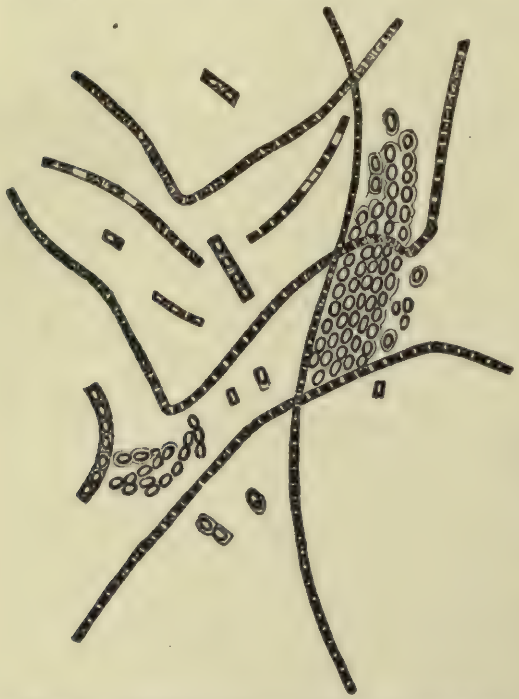

FIG. 99.—Bacillus anthracis, from a culture, showing formation of spores. × 1,000. (Klein.)

The anthrax bacillus *stains* readily with the aniline colors and also by Gram's method, when not left too long in the decolorizing iodine solution. Löffler's solution of methylene blue is an especially good staining fluid for this as well as for many other bacilli. Bismarck brown is well adapted for specimens which are to be photographed, and also for permanent preparations, as it is less liable to fade than the blue and some other aniline colors.

Biological Characters.—The anthrax bacillus is *aërobic*, but not strictly so, as is shown by the fact that it grows to the bottom of the line of puncture in stab cultures in solid media. It is *non-motile*, and is distinguished by this character from certain common bacilli resembling it in morphology—Bacillus subtilis—which were frequently confounded with it in the earlier days of bacteriological investigation.

The anthrax bacillus grows in a variety of nutrient media at a temperature of 20° to 38° C. Development ceases at temperatures below 12° C. or above 45° C.

This bacillus grows best in neutral or slightly alkaline media, and its development is arrested by a decidedly acid reaction of the culture medium. It may be cultivated in infusions of flesh or of various vegetables, in diluted urine, in milk, etc.

In *gelatin plate cultures* small, white, opaque colonies are developed in from twenty-four to thirty-six hours, which under the microscope are seen to be somewhat irregular in outline and of a greenish tint; later the colonies spread out upon the surface of the gelatin, and the darker central portion is surrounded by a brownish mass of wavy filaments, which are associated in tangled bundles. Mycelial-like outgrowths from the periphery of the colony may often be seen extending into the surrounding gelatin. At the end of two or three days liquefaction of the gelatin commences, and the colony is soon surrounded by the liquefied medium, upon the surface of which it floats as an irregular white pellicle. In *gelatin stab cultures* growth occurs all along the line of puncture as a white central thread, from which lateral thread-like ramifications extend into the culture medium. At the end of two or three days liquefaction of the culture medium commences near the surface, where the development has been most abundant. At first a pasty, white mass is formed, but as liquefaction progresses the upper part of the liquefied gelatin becomes transparent from the subsidence of the motionless bacilli, and these are seen upon the surface of the non-liquefied portion of the medium in the form of cloudy, white masses, while below the line of liquefaction the characteristic branching growth may still be seen along the line of puncture.

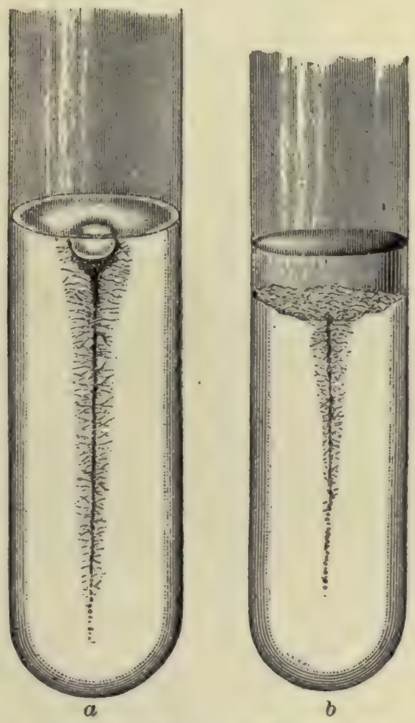

FIG. 100.—Culture of Bacillus anthracis in nutrient gelatin: *a*, end of four days; *b*, end of eight days. (Baumgarten.)

In *agar plate cultures*, in the incubating oven at 35° to 37° C., colonies are developed within twenty-four hours, which under the microscope are seen to be made up of interlaced filaments and are very characteristic and beautiful. Upon the surface of nutrient agar a grayish-white layer is formed, which may be removed in ribbon-like strips; and in stick cultures in this medium a branching growth is seen, like that in gelatin, but without liquefaction. The addition of a small quantity of agar to a gelatin medium prevents liquefaction of the gelatin (Flügge).

Upon *blood serum* a rather thick, white layer is formed and liquefaction slowly occurs.

Upon *potato* the growth is abundant as a rather dry, grayish-white layer, of limited extent, having a somewhat rough surface and irregular margins.

Spores are formed only in the free presence of oxygen, as in surface cultures upon potato or nutrient agar, or in shallow cultures in liquid media, and at a temperature of 20° to 35° C. They are not formed during the development of the bacilli in the bodies of living animals, but after the death of the animal the bacillus continues to multiply for a time, and spores may be formed where the fluids containing it come in contact with the air—as, for example, in bloody discharges from the nostrils or from the bowels of the dead animal.

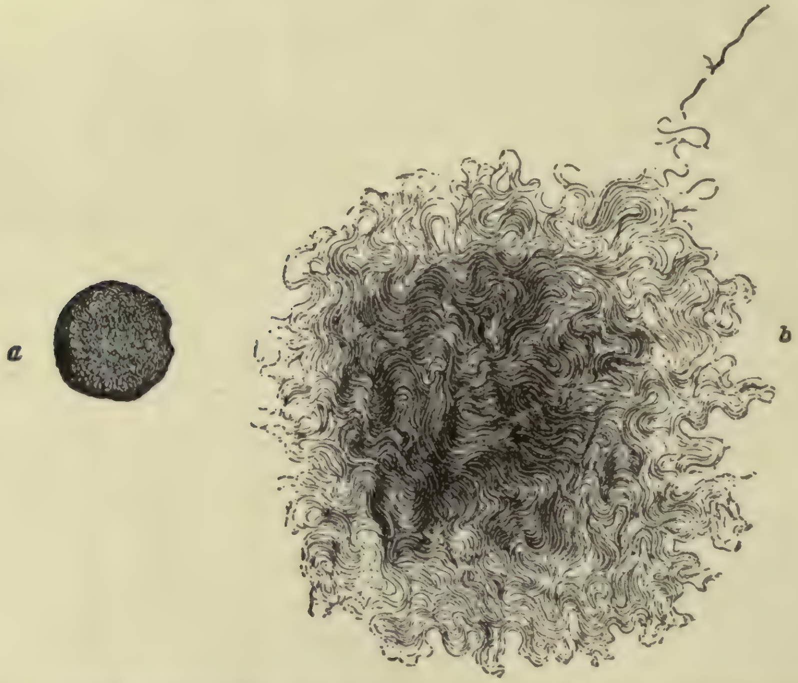

FIG. 101.—Colonies of Bacillus anthracis upon gelatin plates: *a*, at end of twenty-four hours; *b*, at end of forty-eight hours. × 80. (Flügge.)

Varieties incapable of spore production have been produced artificially, by several bacteriologists, by cultivating the bacillus under unfavorable conditions. Roux was able to produce a sporeless variety by successive cultivation in media containing a small quantity of carbolic acid—1 : 1,000.

Varieties differing in their pathogenic power may also be produced by cultivation under unfavorable conditions. Thus Pasteur produced an "attenuated virus" by keeping his cultures for a considerable time before replanting them upon fresh soil, and supposed the effect was due to the action of atmospheric oxygen. It seems probable that it was rather due to the deleterious action of its own products of growth present in the culture media. It has been shown by Chamberlain and Roux that cultivation in the presence of certain chemical substances added to the culture medium—*e.g.*, bichromate of potassium 0.01 per cent—causes an attenuation of virulence. The same result occurs when cultures are subjected to a temperature a little below that which is fatal to the bacillus—50° C. for eighteen minutes (Chauveau); 42.5° C. for two or three weeks (Koch). Attenuation of pathogenic virulence is also effected by cultivation in the body of a non-susceptible animal, like the frog (Lubarsch, Petruschky); or in the blood of a rat (Behring); by exposure to sunlight (Arloing); and by compressed air (Chauveau).

Anthrax spores may be preserved in a desiccated condition for years without losing their vitality or pathogenic virulence when inoculated into susceptible animals. They also resist a comparatively high temperature. Thus Koch and Wolffhügel found that dry spores exposed in dry air required a temperature of 140° C., maintained for three hours, to insure their destruction. But spores suspended in a liquid are destroyed in four minutes by the boiling temperature, 100° C. (writer's determination).

The bacilli, in the absence of spores, according to Chauveau, are destroyed in ten minutes by a temperature of 54° C.

For the action of various antiseptic and germicidal agents upon this bacillus we must refer to the sections especially devoted to this subject (Part Second).

Toussaint, by injecting filtered anthrax blood into animals, obtained evidence that it contained some toxic substance which in his experiments gave rise to local inflammation without any noticeable general symptoms. More recent investigations show that a poisonous substance is formed during the growth of the anthrax bacillus, and that cultures containing this toxin, from which the bacilli have been removed by filtration through porcelain, produce immunity when injected into susceptible animals, similar to that resulting from inoculations with an attenuated virus. It is probable that the pathogenic power of the anthrax bacillus depends largely upon the presence of this toxin, and that the essential difference between virulent and attenuated varieties depends upon the more abundant production of this toxic substance by the former. It has also been shown that virulent cultures produce a larger quantity of acid than those which have been attenuated by any of the agencies above mentioned (Behring).

Pathogenesis.—The anthrax bacillus is pathogenic for cattle, sheep, horses, rabbits, guinea-pigs, and mice. White rats, dogs, and frogs are immune, as is also the Algerian race of sheep. The sparrow is susceptible to general infection, but chickens, under normal conditions, are not. Young animals are, as a rule, more susceptible than adults of the same species. Man does not belong among the most susceptible animals, but is subject to local infection as a result of accidental inoculation—malignant pustule—and to pulmonic anthrax from breathing air, containing spores of the anthrax bacillus, during the sorting of wool or hair from infected animals. In animals which have a partial immunity, natural or acquired, as a result of inoculations with attenuated virus, the subcutaneous introduction of virulent cultures may give rise to a limited local inflammatory process, with effusion of bloody serum in which the bacillus is found in considerable numbers ; but the blood is not invaded, and the animal, after some slight symptoms of indisposition, recovers. In susceptible animals injections beneath the skin or into a vein give rise to general infection, and the bacilli multiply rapidly in the circulating fluid. Death occurs in mice within twenty-four hours, and in rabbits, as a rule, in less than forty-eight hours. The blood of the heart and large vessels may be found, in an autopsy made immediately after death, to contain comparatively few bacilli ; but in the capillaries of the various organs, and especially in the greatly enlarged spleen, in the liver, the kidneys, and the lungs, they will be found in great numbers, and well-stained sections of these organs will give an astonishing picture under the microscope, which the student should not fail to see in preparations made by himself. The capillaries in many places will be found stuffed full of bacilli ; or they may even be rup-

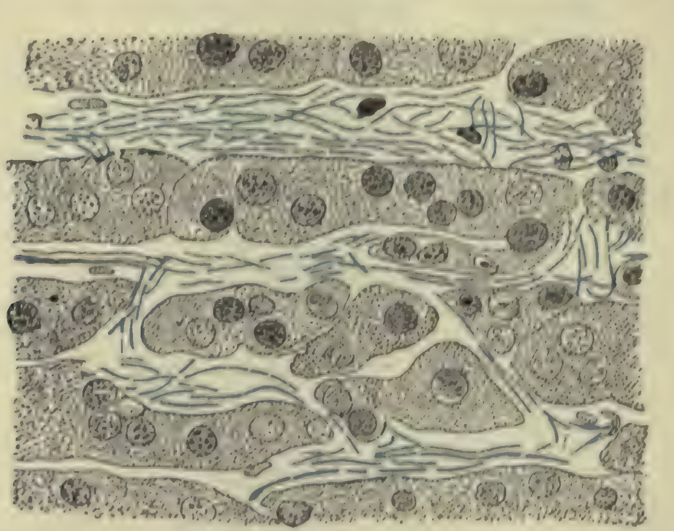

FIG. 102.—Bacillus anthracis in liver of mouse. × 700. (Flügge.)

tured as a result of the distention, and the bacilli, together with escaped blood corpuscles, will be seen in the surrounding tissues. In the kidneys the glomeruli, especially, appear as if injected with colored threads, and by rupture these may find their way into the uriniferous tubules.

These appearances and the general symptoms indicate that the disease produced by the introduction of this bacillus into the bodies of susceptible animals is a genuine septicæmia. As in other forms of septicæmia, the spleen is found to be greatly enlarged; it has a dark color and is soft and friable. With this exception the organs present no notable changes, although the liver is apt to be somewhat enlarged. In the guinea-pig an extensive inflammatory œdema, extending from the point of inoculation to the most dependent parts of the body, is developed; the subcutaneous connective tissue is infiltrated with bloody serum and has a gelatinous appearance. This animal comes next to the mouse in susceptibility, and cultures which are attenuated to such an extent that they will not kill a rabbit or a sheep may still kill a guinea-pig; or, if not, may kill a mouse. Pasteur has shown that the pathogenic power of the bacillus may be reëstablished by inoculations into susceptible animals, and that an attenuated culture which will not kill an adult guinea-pig may be fatal to a very young animal of this species, and that cultures from the blood of this will have an increased pathogenic virulence.

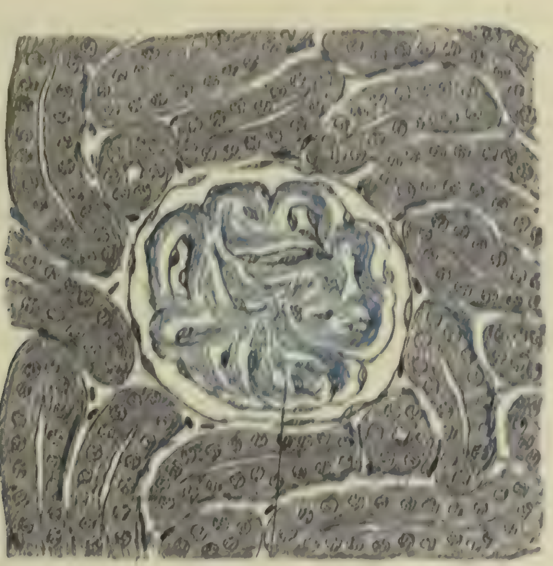

FIG. 103.—Bacillus anthracis in kidney of rabbit. × 400. (Baumgarten.)

Very minute quantities of a virulent culture are infallibly fatal to these most susceptible animals, but for rabbits and other less susceptible animals the quantity injected influences the result, and recovery may occur after subcutaneous or intravenous injection of a very small number of bacilli.

Infection in cattle and sheep commonly results from the ingestion of spores while grazing in infected pastures. The bacillus itself, in the absence of spores, is destroyed in the stomach. While spores are not formed in the bodies of living animals, their discharges contain the bacillus, and this is able to multiply in them and to form spores upon the surface of the ground when temperature conditions are favorable. It is probable that this is the usual way in which pastures become infected, and that the bloody discharges from the bladder and bowels of animals suffering from the disease furnish a nidus for the external development of these reproductive elements; as also do the fluids escaping from the bodies of dead animals. And possibly, under specially favorable conditions, the bacillus may lead a saprophytic existence for a considerable time in the superficial layers of the soil.

Buchner has shown by experiment that infection in animals may result from respiring air in which anthrax spores are in suspension in the form of dust; and in man this mode of infection occurs in the so-called wool-sorters' disease.

The question of the passage of the anthrax bacillus from the mother to the fœtus in pregnant females has received considerable attention. That this may occur is now generally admitted, and appears to be established by the investigations of Strauss and Chamberlain, Morisani, and others. That it does not always occur is shown, however, by the researches of other bacteriologists, and especially by those of Wolff.

Sirena and Scagliosi (1894) report, as the result of extended experiments made by them, that anthrax spores may survive in distilled water for twenty months; in moist or dry earth for two years and nine months; in sea-water for one year and seven months; in sewage nearly sixteen months.

Marmier (1895) has made an extended experimental research to determine the nature of the specific toxin of the anthrax bacillus. This he obtains from cultures, at a low temperature, in media containing peptone and glycerin. It has not the reactions of an albuminoid body and is not destroyed by a temperature of 100° C. In comparatively large doses it kills animals susceptible to anthrax, and by the administration of smaller doses immunity may be established in such animals. This toxin is contained in the bacterial cells, and is obtained by subjecting these to the action of alcohol, or from the filtrate when cultures are made at a low temperature in a medium containing peptone. It has not, however, been obtained in a pure form, and its exact nature has not been determined.

IX.

THE BACILLUS OF TYPHOID FEVER.

NUMEROUS researches support the view that the bacillus described by Eberth in 1880 bears an etiological relation to typhoid fever—*typhus abdominalis* of German authors; and pathologists have accepted this bacillus as the veritable "germ" of typhoid fever, notwithstanding the fact that the final proof that such is the case is still wanting.

This final proof would consist in the production in man or in one of the lower animals of the specific morbid phenomena which characterize the disease in question, by the introduction of pure cultures of the bacillus into the body of a healthy individual. Evidently it is impracticable to make the test upon man, and thus far we have no satisfactory evidence that any one of the lower animals is subject to the disease as it manifests itself in man. The experiments of Fränkel and Simmonds show, however, that this bacillus is pathogenic for the mouse and the rabbit. We shall refer to the experiments of these authors later.

Before the publication of Eberth's first paper Koch had observed this bacillus in sections made from the spleen and liver of typhoid cases, and had made photomicrographs from these sections. His name is, therefore, frequently associated with that of Eberth as one of the discoverers of the typhoid bacillus. Other investigators had no doubt previously observed the same organism, but some of them had improperly described it as a micrococcus. Such a mistake is easily made when the examination is made with a low power; even with a moderately high power the closely crowded colonies look like masses of micrococci, and it is only by focussing carefully upon the scattered organisms on the outer margin of a colony that the oval or rod-like form can be recognized.

Several observers had noted the presence of microörganisms in the lesions of typhoid fever prior to the publication of Eberth's paper, and Browicz in 1875, and Fischel in 1878, had recognized the presence of oval organisms in the spleen which were probably identical with the bacillus of Eberth.

The researches of Gaffky (1884) strongly support the view that the bacillus under consideration bears a causal relation to typhoid fever. Eberth was only successful in finding the bacillus in the lymphatic glands or in the spleen in eighteen cases out of forty in which he searched for it. On the other hand, he failed to find it in eleven cases of various nature—partly infectious processes—and in thirteen cases of tuberculosis in which the lymphatic glands were involved, and in several of which there was ulceration of the mucous membrane of the intestine.

Koch, independently of Eberth and before the publication of his first paper, had found the same bacillus in about half of the cases examined by him, and had pointed out the fact that they were located in the deeper parts of the intestinal mucous membrane, beyond the limits of necrotic changes, and also in the spleen, whereas the long, slender bacillus of Klebs was found only in the necrosed portions of the intestinal mucous membrane.

The researches of W. Meyer (1881) gave a larger proportion of successful results. This author confined his attention chiefly to the swollen plaques of Peyer and follicles of the intestine which had not yet undergone ulceration. The short bacillus which had been described by Eberth and Koch was found in sixteen out of twenty cases examined. The observations of this author are in accord with those of Eberth as to the presence of the bacillus in greater abundance in cases of typhoid which had proved fatal at an early date.

The fact that in these earlier researches the bacilli were not found in a considerable proportion of the cases examined is by no means fatal to the view that they bear an etiological relation to the disease. As Gaffky says in his paper referred to :

"This circumstance admits of two explanations. Either in those cases in which the bacillus has been sought with negative results they may have perished collectively, before the disease process which thev had induced had run its course ; or the proof of the presence of bacilli was wanting only on account of the technical difficulties which attend the finding of isolated colonies."

Gaffky's own researches indicate that the latter explanation is the correct one.

In twenty-eight cases examined by this author characteristic colonies of the bacillus were found in all but two. In one of these, one hundred and forty-six sections from the spleen, liver, and kidneys were examined without finding a single colony, and in the other a like result attended the examination of sixty-two sections from the spleen and twenty-one sections from the liver. In the first of these cases, however, numerous colonies were found in recent ulcers of the intestinal mucous membrane, deeply located in that portion of the tissue which was still intact. These recent ulcers were in the neighborhood of old ulcers and are supposed to have indicated a relapse of the specific process. In the second case the negative result is thought by Gaffky to have been not at all surprising, as the patient died at the end of the fourth week of sickness, not directly from the typhoid process, but as a result of perforation of the intestine.

Gaffky has further shown that in those cases in which colonies are not found in the spleen, or in which they are extremely rare, the presence of the bacillus may be demonstrated by cultivation; and that, when proper precautions are taken, pure cultures of the bacillus may always be obtained from the spleen of a typhoid case. Hein has been able to demonstrate the presence of the bacillus and to start pure cultures from material drawn from the spleen of a living patient by means of a hypodermatic syringe. Philopowicz has reported his success in obtaining cultures of the bacillus by the same method.

The fact that a failure to demonstrate the presence of microörganisms by a microscopic examination cannot be taken as proof of their absence from an organ, is well illustrated by a case (No. 18) in which the bacillus was obtained by Gaffky from the spleen and also from the liver, in pure cultures; whereas in cover-glass preparations made from the same spleen he failed to find a single rod, and more than one hundred sections of the spleen were examined before he found a colony.

To obtain pure cultures from the spleen Gaffky first carefully washes the organ with a solution of mercuric chloride, 1:1,000. A long incision is then made through the capsule with a knife sterilized by heat. A second incision is made in this with a second sterilized knife, and a third knife is used to make a still deeper incision in the same track. By this means the danger of conveying organisms from the surface to the interior of the organ is avoided. From the bottom of this incision a little of the soft splenic tissue is taken up on a sterilized platinum needle, and this is plunged into the solid culture medium, or drawn along the surface of the same, or added to liquefied gelatin and poured upon a glass plate. The colonies develop, in an incubating oven, in the course of twenty-four to forty-eight hours.

Gaffky has also shown that the bacillus is present in the liver, in the mesenteric glands, and, in a certain proportion of cases at least, in the kidneys, in which it was found in three cases out of seven.

The appearance of the colonies in stained sections of the spleen is shown in Figs. 104 and 105. Two colonies are seen in Fig. 104 (at *a*, *a*) as they appear under a low power—about sixty diameters. In Fig. 105 one of the colonies is seen more highly magnified—about five hundred diameters.

Fränkel and Simmonds have demonstrated that the bacilli multiply in the spleen after death, and that numerous colonies may be found in portions of the organ which have been kept for twenty-four to forty-eight hours before they were placed in alcohol, when other pieces from the same spleen placed in alcohol soon after the death of the patient show but few colonies or none at all.

This observation does not in any way weaken the evidence as to the etiological rôle of the bacillus, but simply shows that dead animal matter is a suitable nidus for the typhoid germ—a fact which has been repeatedly demonstrated by epidemiologists and insisted upon by sanitarians.

The authors last referred to confirm Gaffky as regards the constant presence of the bacillus in the spleen. In twenty-nine cases they obtained it by plate cultures twenty-five times, and remark that in the four cases attended with a negative result this result is not at all surprising, inasmuch as the typhoid process had terminated and death resulted from complications.

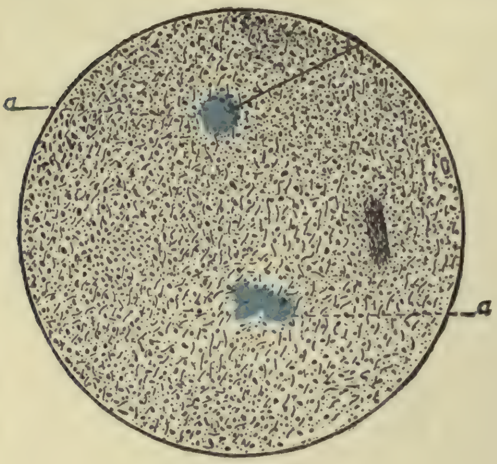

FIG. 104.

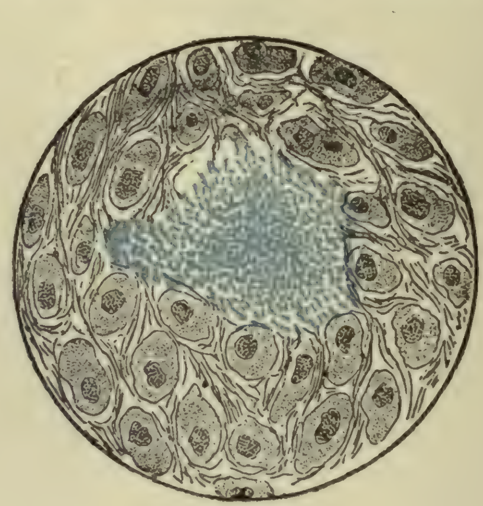

FIG. 105.

Gaffky did not succeed in obtaining cultures from the blood of typhoid-fever patients, and concludes from his researches that if the bacilli are present in the circulating fluid it must be in very small numbers. He remarks that possibly the result would be different if the blood were drawn directly from a vein instead of from the capillaries of the skin. Fränkel and Simmonds also report that gelatin, to which blood drawn from the forefinger of typical cases had been added, remained sterile when poured upon plates in the usual manner—Koch's method. The blood was obtained from six different individuals, all in an early stage of the disease—the second to the third week. A similar experiment made with blood obtained, post mortem, from the large veins or from the heart, also gave a negative result in every instance save one. In the exceptional case a single colony developed upon the plate. In view of these results we are inclined to attribute the successful attempts reported by some of the earlier experimenters (Letzerich, Almquist, Maragliano) to accidental contamination and imperfect methods of research. The more recent work of Tayon does not inspire any greater confidence. This author obtained cultures in bouillon by inoculating it with blood drawn from a typhoid patient, and found that these were fatal, *in a few hours*, to guinea-pigs, when injected into the peritoneal cavity. The lesions observed are said to have resembled those of typhoid fever—congestion and tumefaction of Peyer's plaques and of the mesenteric glands, congestion of the liver, the kidneys, etc.

The presence of the bacillus of Eberth in the alvine evacuations of typhoid patients has been demonstrated by Pfeiffer and by Fränkel and Simmonds. This demonstration is evidently not an easy matter, for while the bacilli are probably always present in some portion of the intestine during the progress of the disease, it does not follow that they are present in every portion of the intestinal contents. As only a very small amount of material is used in making plate cultures, and as there are at all times a multitude of bacteria of various species in the smallest portion of fæcal matter, it is not to be expected that the typhoid bacillus will be found upon every plate. Fränkel and Simmonds made eleven attempts to obtain the bacillus by the plate method, using three plates each time, as is customary with those who adhere strictly to the directions of the master, and were successful in obtaining the bacillus in three instances—in two in great numbers and in the third in a very limited number of colonies.

The numerous attempts which have been made to communicate typhoid fever to the lower animals have given a negative result in every instance. Murchison, in 1867, fed typhoid-fever discharges to swine, and Klein has made numerous experiments of the same kind upon apes, dogs, cats, guinea-pigs, rabbits, and white mice, without result. Birch-Hirschfeld, in 1874, by feeding large quantities of typhoid stools to rabbits, produced in some of them symptoms which in some respects resembled those of typhoid; but these experiments were repeated by Bahrdt upon ten rabbits with an entirely negative result. Von Motschukoffsky met with no better success in his attempts to induce the disease by injecting blood from typhoid patients into apes, rabbits, dogs, and cats. Walder also experimented with fresh and with putrid discharges from typhoid patients, and with blood taken from the body after death, feeding this material to calves, dogs, cats, rabbits, and fowls, without obtaining any positive results. Klebs has also made numerous experiments of a similar nature, and in a single instance found in a rabbit, which died forty-seven hours after receiving a subcutaneous injection of a culture fluid containing his "typhoid bacillus," pathological lesions resembling those of typhoid.

Eberth and Gaffky very properly decline to attach any importance to this solitary case, in which, as the first-named writer remarks, a different explanation is possible, and the possibility of an intestinal mycosis not typhoid in its nature must be considered.

Gaffky has also made numerous attempts to induce typhoid symptoms in animals by means of pure cultures of Eberth's bacillus, given with their food or injected into the peritoneal cavity or subcutaneously. The first experiments were made upon five Java apes. For a considerable time these animals were fed daily with pure cultures containing spores. The temperature of the animals was taken twice daily. The result was entirely negative. No better success attended the experiments upon rabbits (16), guinea-pigs (13), white rats (7), house mice (11), field mice (4), pigeons (2), one hen and a calf.

Cornil and Babes report a similar negative result from pure cultures of the typhoid bacillus injected into the peritoneal cavity and into the duodenum in rabbits and guinea-pigs.

Fränkel and Simmonds have made an extended series of experiments upon guinea-pigs, rabbits, and mice, and have shown that pure cultures of the bacillus of Eberth injected into the last-mentioned animals—mice and rabbits—may induce death, and that the bacillus may again be obtained in pure cultures from their organs. It is not claimed that the animals suffer an attack of typhoid fever as the result of these injections, but that their death is due to the introduction into their bodies of the typhoid bacillus, and that this bacillus is thereby proved to be pathogenic.

BACILLUS TYPHI ABDOMINALIS.

Synonyms.—Bacillus typhosus; Typhus bacillus.

Eberth (1880 and 1881) demonstrated the presence of this bacillus in the spleen and diseased glands of the intestine in typhoid cadavers. Gaffky (1884) first obtained it in pure cultures from the same source and determined its principal biological characters.

It is found, in the form of small, scattered colonies, in the spleen, the liver, the glands of the mesentery, the diseased intestinal glands, and in smaller numbers in the kidneys, in fatal cases of typhoid fever; it has also been obtained, by puncture, from the spleen during life, from the alvine discharges of the sick, and rarely from the urine. It is not found in the blood of the general circulation, unless, possibly, in rare cases and in small numbers.

Morphology.—Bacilli, usually one to three μ in length and about

0.5 to 0.8 μ broad, with rounded ends; may also grow out into long threads, especially upon the surface of cooked potato. The dimensions of the rods differ considerably in different media. Spherical or oval refractive granules are often seen at the extremities of the rods, especially in potato cultures kept in the incubating oven; these are not reproductive spores, as was at first supposed. The bacilli have numerous flagella arranged around the periphery of the cells—usually from five to twenty, but many short rods have but a single terminal flagellum. These flagella are spiral in form, about 0.1 μ in thickness, and from three to five times as long as the rods (Babes).

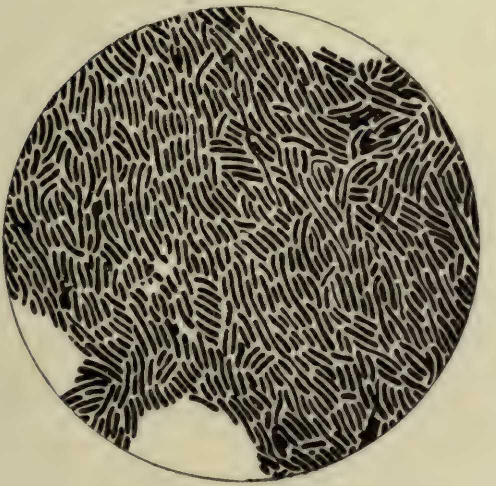

Fig. 106.

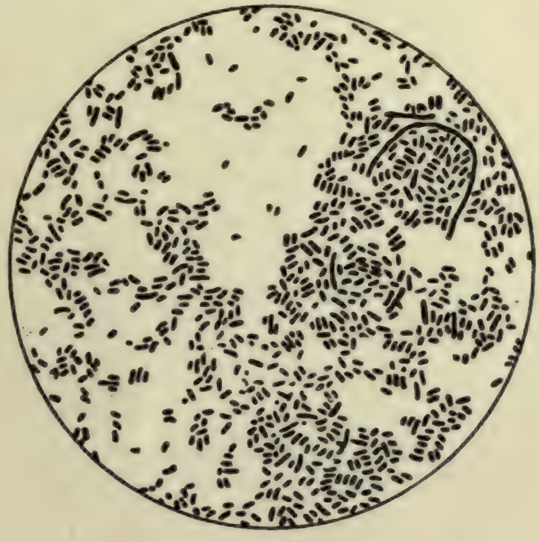

Fig. 107.

Fig. 106.—Bacillus typhi abdominalis, from single gelatin colony. × 1,000. From a photomicrograph. (Fränkel and Pfeiffer.)

Fig. 107.—Bacillus typhi abdominalis, from single gelatin colony. × 1,000. From a photomicrograph. (Sternberg.)

In stained preparations unstained "vacuoles" may often be seen at the margins of the rods, either along the sides or at the ends; these appear to be due to a retraction of the protoplasm from the cell membrane.

The typhoid bacillus *stains* with the aniline colors, but more slowly than many other bacteria, and easily parts with its color when treated with decolorizing agents—*e.g.*, iodine solution as employed in Gram's method. Löffler's solution of methylene blue is an excellent staining agent for this bacillus, but permanent preparations fade out after a time; fuchsin, gentian violet, or Bismarck brown, in aqueous solution, may also be used. The flagella may be demonstrated by Löffler's method of staining (p. 32).

To stain the bacillus in sections of the spleen, etc., it is best to leave these in Löffler's methylene blue solution or in the carbolfuchsin solution of Ziehl for twelve hours or more; or the aniline-fuchsin solution may be used. The sections should be washed in distilled water only, when Ziehl's solution is used, or with a very dilute solution of acetic acid when Ehrlich's tubercle stain is employed (Baumgarten).

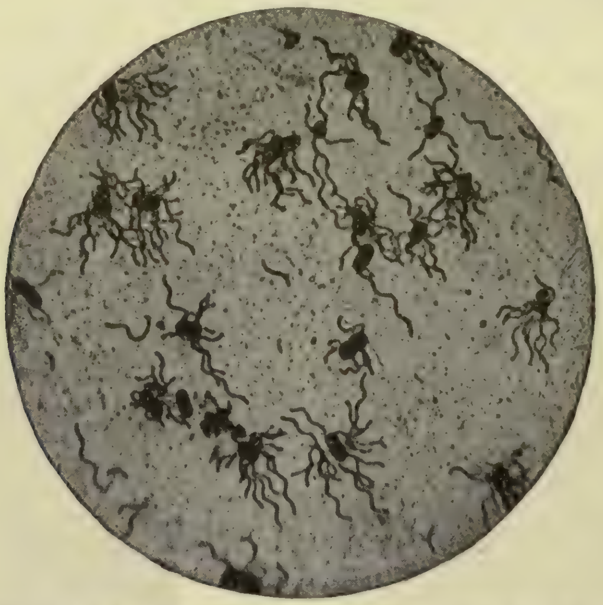

Fig. 108.—Bacillus typhi abdominalis, stained by Löffler's method, showing flagella. × 1,000. From a photomicrograph by Fränkel and Pfeiffer.

Biological Characters.—The typhoid bacillus is a *motile, aërobic, non-liquefying* bacillus, which grows readily in a variety of culture media at the "room temperature." Although it grows most abundantly in the presence of free oxygen, it may also develop in its absence, and is consequently a *facultative anaërobic.*

In *gelatin plate cultures* small, white colonies are developed at the end of thirty-six to forty-eight hours, which under the microscope are seen to be somewhat irregular in outline and of a spherical, oval, or long-oval form; these have by transmitted light a slightly granular appearance and a yellowish-brown color. At the end of three or four days the colonies upon the surface of the gelatin form a grayish-white layer of one to two millimetres in diameter, with more or less irregular margins, and, when developed from deep colonies, with an opaque central nucleus. These colonies, by transmitted light, have a yellowish-brown color towards the centre, where they are thickest, while the margins are colorless and transparent; the surface is com-

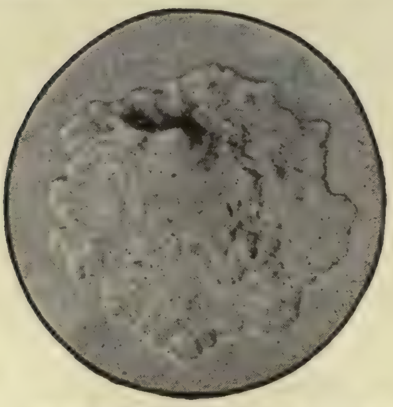

Fig. 109.—Single colony of Bacillus typhi abdominalis, in nutrient gelatin. (×?) From a photograph by Roux.

monly marked with a network of lines and furrows. Stab cultures in ten-per-cent gelatin, at 18° to 20° C., at the end of three days show upon the surface a whitish, semi-transparent layer, with sharply defined margins and irregular outline, which has a shining, pearly lustre; and along the line of puncture a grayish-white growth, made up of crowded colonies, which are larger and more distinct at the bottom of the line of growth. Upon *nutrient agar*, at a temperature of 35° to 37° C., the growth is more rapid and forms a whitish, semi-transparent layer. The cultures give off a faint putrefactive odor. The growth upon blood serum is rather scanty, in the form of transparent, shining patches along the line of inoculation.

The typhoid bacillus develops abundantly in *milk*, in which fluid it produces an acid reaction; it also grows in various vegetable infusions and in bouillon.

None of the above characters of growth are distinctive, as certain common bacilli found in normal fæces present a very similar appearance when cultivated in the same media.

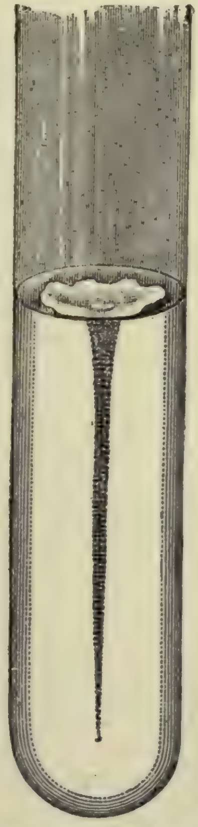

FIG. 110.—Bacillus typhi abdominalis; stick culture in nutrient gelatin, eighth day at 16°-20° C. (Baumgarten.)

The growth of this bacillus upon *potato* is an important character, as was first pointed out by Gaffky. In the incubating oven at the end of forty-eight hours, or at the room temperature in three or four days, the surface of the potato has a moist, shining appearance, but there is no visible growth such as is produced by many other bacteria upon this medium. A simple inspection would lead to the belief that no growth had occurred; but if with a platinum needle a little material is scraped from any portion of the shining surface and a stained preparation is made from it, numerous bacilli will be seen, some of which are likely to be in the form of quite long threads, while others are short and have rounded extremities. This "invisible growth" has been shown by the researches of Buchner and others to be most characteristic upon potatoes having a decidedly acid reaction, as is usually the case. When cultivated upon potatoes having an alkaline reaction a thin, visible film of a yellowish-brown color and of limited extent may be developed. Inasmuch as several common and widely distributed bacteria closely resemble the typhoid bacillus in form and in their growth in nutrient gelatin, this character of invisible growth upon potato is very important for its differentiation, especially as the common bacilli referred to—Bacillus coli communis, bacillus of Emmerich—produce a very distinct and rather thick, yellowish-white mass upon the surface of potato. But recent researches show that this invisible growth, although not a common character, does not belong exclusively to the typhoid bacillus (Babes).

This bacillus in its development in culture media produces acids—according to Brieger small quantities of volatile fat acids, and, in presence of grape sugar, lactic acid. It also grows readily in a decidedly acid medium, and this character has been employed as a test for differentiating it from other similar bacilli; but some of these also grow in a decidedly acid medium, and too much reliance cannot be placed upon this test.

Brieger has shown that indol is not produced in cultures of the typhoid bacillus, and Kitasato has proposed to use the indol test for differentiating this from other similar bacilli which are said, as a rule, to give the indol reaction. This test consists in the addition to ten cubic centimetres of a bouillon culture which has been in the incubating oven for twenty-four hours, of one cubic centimetre of a solution of sodium nitrite (0.02 gramme to one hundred cubic centimetres of distilled water), together with a few drops of concentrated sulphuric acid. If indol is present a red color is developed.

None of the above-mentioned tests are entirely reliable, but, taken together with the morphological and biological characters above described, they may enable the bacteriological expert to give a tolerably confident opinion as to the presence of this bacillus in a water supply suspected of contamination, etc. And when a bacillus having these characters is obtained in a pure culture from the spleen of a typhoid cadaver the student may be very sure that he has the typhoid bacillus. But in the presence of various similar bacilli, as in fæces, very careful comparative researches will be required to determine in a definite manner that a non-liquefying bacillus obtained in pure cultures by the plate method is really the one now under consideration—especially so as the cultures of the typhoid bacillus in the same medium may differ considerably at different times, and a number of bacilli are known which resemble it so closely that it is still uncertain whether they are to be considered as varieties of the typhoid bacillus or as distinct species. Thus Babes, in an extended research, found in the organs of typhoid cases, associated with the true typhoid bacillus, other bacilli or varieties very closely resembling it. He has also described three varieties (?), obtained by him from other sources, which could only be differentiated from the true typhoid bacillus by very careful comparison of cultures made side by side in various media.

Cassedebat, also, in an extended examination of the river water at Marseilles with reference to the presence of the typhoid bacillus, found three species which very closely resembled it, but which by careful comparison were shown to present slight but constant differences in their biological characters. He was not able to find the true typhoid bacillus, and his researches, together with those of Babes and other recent investigators, make it appear probable that numerous mistakes have been made by bacteriologists who have reported the finding of the typhoid bacillus in river and well water, in fæces, etc., and who have depended mainly upon the character of invisible growth upon potato in making their diagnosis. Cassedebat states that all three of his pseudo-typhoid bacilli corresponded in their growth upon potato with the bacillus of Eberth. They also corresponded in their growth on gelatin, agar-agar, and blood serum, which, as heretofore remarked, has no characteristic features. They all gave a negative indol reaction. Like the typhoid bacillus, they grew in milk without causing coagulation of the casein, but two of them produced an alkaline reaction in this fluid, while the third corresponded with the typhoid bacillus in producing a decided acid reaction. Differences were also observed in bouillon cultures, and in bouillon and milk to which various aniline colors had been added, as recommended by Holz.

Whether the typhoid bacillus, as obtained from the spleen of a typhoid cadaver, is in truth specifically distinct from these similar bacilli, or whether they are all varieties of the same species, resulting from modifications in their biological characters acquired during their continuous development under different conditions, is an unsettled question. But, in view of the experimental evidence now available, there is nothing improbable in the supposition that they are simply varieties, and that, as the result of a saprophytic mode of life, this bacillus may undergo more or less permanent modifications.

In the writer's experiments (1887) the thermal death-point of the typhoid bacillus was found to be 56° C., the time of exposure being ten minutes ; and potato cultures containing the refractive granules described by Gaffky as spores were found to be infallibly destroyed by a temperature of 60° C. This result has been confirmed by Buchner (1888) and by Janowsky (1890), and the inference seems justified that these granules are not reproductive bodies, as was at first believed ; for spores are distinguished by their great resistance to heat and other destructive agencies. According to Buchner, the bacilli containing these refractive granules are even less resistant than fresh cultures in which they are not present, and he is disposed to look upon them as representing a degeneration of the protoplasm of the cells. They do not stain by the methods which are successful in staining the spores of other bacilli, and, in short, present none of the characters which distinguish spores, except the form and high refractive power.

The typhoid bacillus retains its vitality for many months in cultures; the writer has preserved bouillon cultures for more than a year in hermetically sealed tubes, and has found that development promptly occurred in nutrient gelatin inoculated from these. Dried upon a cover glass, it may grow in a suitable medium after having been preserved for eight to ten weeks (Pfuhl). When added to sterilized distilled water it may retain its vitality for more than four weeks (Bolton), (forty days Cassedebat), and in sterilized sea-water for ten days (De Giaxa). Added to putrefying fæces it may preserve its vitality for several months (Ufflemann), in typhoid stools for three months (Karlinski), and in earth upon which bouillon cultures had been poured for five and one-half months (Grancher and Deschamps).

In hanging-drop cultures this bacillus may be seen to exhibit very active movements, the shorter rods rapidly crossing the field with a darting or to-and-fro, progressive motion, while longer filaments move in a serpentine manner.

In addition to the volatile fat acids which, according to Brieger, are formed in small amounts in cultures of the typhoid bacillus, and to lactic acid formed in solutions containing grape sugar, a basic substance possessing toxic properties has been isolated by the chemist named—his *typhotoxine* ($C_7H_{17}NO_2$). Brieger supposes that other basic substances are likewise formed, but believes this to be the specific product to which the pathogenic action of the bacillus is due. It is a strongly alkaline base, which produces in mice and guinea-pigs salivation, paralysis, dilated pupils, diarrhœa, and death.

Numerous experiments have been made to determine the amounts of various germicidal agents required to destroy the vitality of this bacillus, and the action of antiseptics in restraining its development. For the results of these experiments the reader is referred to the sections in Part Second relating to the action of antiseptics and disinfectants.

Pathogenesis.—The very numerous experiments which have been made on the lower animals have not been successful in producing in any one of them a typical typhoid process. Nor is this surprising, in view of the fact that, so far as is known, no one of them is liable to contract the disease, as man does, by the use of infected food or water.

The experiments of Fränkel and Simmonds show that when considerable quantities of a pure culture of this bacillus are injected into the circulation of rabbits through the ear vein, or into the peritoneal cavity of mice, a certain proportion of the inoculated animals die, usually within forty-eight hours, and that the bacillus may be recovered from the various organs, although it is not present in the blood. But death does not always occur from intravenous injections, and subcutaneous or intraperitoneal injections in rabbits are usually without result. Subcutaneous injections in mice proved to be fatal in ten cases out of sixteen inoculated by A. Fränkel. Seitz, by following Koch's method—*i.e.*, by rendering the contents of the stomach alkaline, and arresting intestinal peristalsis by the administration of opium—obtained a fatal result, in a majority of the guinea-pigs experimented upon, from the introduction of ten cubic centimetres of a bouillon culture into the stomach through a pharyngeal catheter. We may remark, with reference to these results, that while they show that cultures of the typhoid bacillus have a certain pathogenic power, they also show that the animals experimented upon frequently recovered after comparatively large doses, and that the typhoid bacillus is not pathogenic in the same sense as are those microörganisms which, when introduced into the body of a susceptible animal in very minute amount, give rise to general infection and death. On the other hand, a fatal result depends upon the quantity of the culture introduced in the first instance, rather than upon the multiplication of the bacillus in the body of the inoculated animal. This view is confirmed by the experiments of Sirotinin, which show not only that a fatal result depends upon the quantity injected, but also that a similar result follows the injection of cultures which have been sterilized by heat or filtration. The pathogenic action, then, depends upon the presence of toxic substances produced during the growth of the bacillus in artificial culture media. The researches of Brieger, heretofore referred to, show the presence in such cultures of a toxic ptomaine—his typhotoxine—to which the pathogenic potency of these

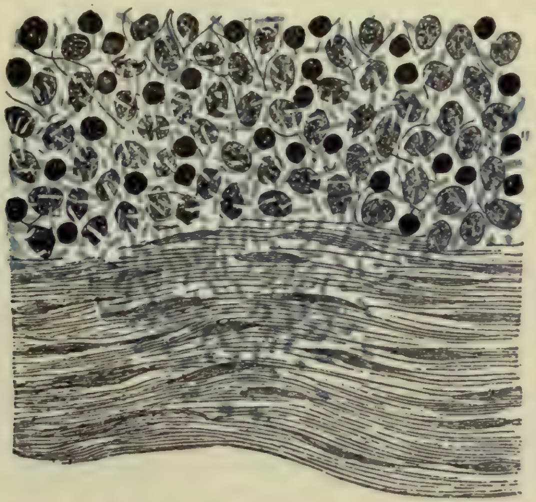

FIG. 111.—Section through wall of intestine, showing invasion by typhoid bacilli. × 950. (Baumgarten.)

cultures appear to be due. White mice and guinea-pigs usually die in from twenty-four to forty-eight hours when inoculated in the cavity of the abdomen with a virulent culture of the typhoid bacillus —0.1 cubic centimetre to 0.5 cubic centimetre of a bouillon culture three days old. According to Kitasato, the virulence of cultures from different cases of typhoid fever varies considerably.

Detection of the Typhoid Bacillus in Water.—The generally recognized fact that typhoid fever is usually contracted by drinking water contaminated by the typhoid bacillus has led to numerous researches having for their object the discovery of a reliable method of detecting this bacillus when present in water in comparatively small numbers in association with the ordinary water bacilli. The use of Koch's plate method, as commonly employed, will not suffice, because the water bacilli present grow more rapidly and cause liquefaction of the gelatin before visible colonies of the typhoid bacillus are formed; and, owing to the relatively small number of typhoid bacilli, these are likely to escape detection. The aim of bacteriologists has, therefore, been to restrain the growth of these common water bacilli by some agent which does not at the same time prevent the development of the typhoid bacillus. Chantemesse and Widal were the first to propose the use of carbolic acid for this purpose. They recommended the addition of 0.25 per cent of this agent to nutrient gelatin; but, according to Kitasato, the development of the typhoid bacillus is restrained by an amount exceeding 0.20 per cent.

Holz prepares an acid medium by adding gelatin (ten per cent) to the juice of raw potatoes, and asserts that while the typhoid bacillus grows luxuriantly in this medium, many other bacilli fail to develop in it. The test is said to be still more reliable if 0.05 per cent of carbolic acid is added to the "potato-gelatin." According to Holz, the addition of more than 0.1 per cent of carbolic acid to nutrient gelatin prevents the free development of the typhoid bacillus.

Thoinot has claimed to be able to obtain the typhoid bacillus from mixed cultures—as, for example, from fæces—by suspending a small amount of material containing it for several hours in a solution containing 0.25 per cent of carbolic acid. While other bacilli are destroyed, the typhoid bacillus is said to survive such exposure.

The method of Parietti has been tested in a practical way by Kamen, and proved to be satisfactory for the detection of the typhoid bacillus in water which was supposed to be the source of a local epidemic of the disease. The following solution is used:

Carbolic acid, ...	5 grammes.
Hydrochloric acid (pure), ...	4 "
Distilled water, ...	100 "

Several test tubes, each of which contains ten cubic centimetres of neutral, sterilized bouillon, are used in the experiment. From three to nine drops of the acid solution are added to each of these, and the tubes are then placed in an incubating oven for twenty-four hours to ascertain whether they are still sterile after this addition. If the bouillon remains clear, from one to ten drops of the suspected water are added to each tube and they are returned to the incubating oven. If at the end of twenty-four hours the bouillon becomes clouded, this is due, according to Parietti, to the presence of the typhoid bacillus, which is then to be obtained in pure cultures by the plate method.

The following method, suggested by Hazen and White, has been tested with favorable results by Foote. This method depends upon the fact that most of the common water bacilli do not grow at a temperature of 40° C., whereas this is a favorable temperature for the development of the typhoid bacillus. A small quantity of the suspected water is added to liquefied nutrient agar in test tubes, and plates are made. These are placed in an incubating oven at 40° C., and the typhoid bacillus, if present, will develop colonies within two or three days. At the ordinary room temperature the more numerous water bacilli would develop upon the same plates so abundantly that it would be difficult to recognize colonies of the typhoid bacillus.

Theobald Smith (*Centralb. f. Bakteriol.*, Bd. xii., page 367), has shown that the typhoid bacillus may be differentiated from other similar bacilli (Bacillus coli communis, bacillus of hog cholera, etc.) by the fact that it does not produce gas in culture media containing sugar—grape sugar, cane sugar, or milk sugar. The medium recommended by Smith for making this test is a peptone-bouillon containing two per cent of grape sugar and made slightly alkaline with carbonate of soda. The liquid becomes clouded throughout at the end of twenty-four hours, but not a trace of gas is developed even after several days. On the other hand, the colon bacillus and other bacilli which closely resemble the typhoid bacillus cause an abundant development of gas in this medium.

The method of Wurtz will be found useful in the detection of colonies of the typhoid bacillus in plate cultures from contaminated water, etc. This consists in the addition to the nutrient medium of lactose (two per cent) and a solution of litmus. When the colonies develop in plates made from this medium the typhoid colonies remain blue, while colonies of the "colon bacillus" have a red color, on account of the development of lactic acid.

Schild (1894) uses a bouillon containing formalin (1:7,000) and claims that the typhoid bacillus fails to grow in this medium, while the bacilli of the colon group multiply in it and cause the medium to become clouded within twenty-four hours. Abel (1894), as a result of extended experiments, arrives at the conclusion that the formalin test cannot be relied upon for distinguishing the typhoid bacillus from certain similar bacilli, which also fail to grow in formalin solution. But, on the other hand, a bacillus which grows in bouillon containing 1 : 7,000 of formalin can be definitely pronounced to be not the typhoid bacillus.

Elsner (1895) recommends the following method for the detection of the typhoid bacillus in water or in fæces: To potato gelatin, prepared by the method of Holz, he added one per cent of potassium iodide. But few species of bacteria will grow in this medium, but Bacillus coli communis grows in it luxuriantly, forming fully developed colonies at the end of twenty-four hours. The typhoid colonies, on the contrary, are only just visible under a low power at the end of twenty-four hours, and at the end of forty-eight hours are seen as small, shining, drop-like, very finely granular colonies. At the same time the colonies of the colon bacillus are much larger, coarsely granular, and of a brownish color. By this method Elsner succeeded in obtaining pure cultures of the typhoid bacillus from the fæces in fifteen out of seventeen cases of typhoid fever, in various stages of the disease. Lazarus (1895) has tested this method and reports that he succeeded without any difficulty in obtaining pure cultures of the typhoid bacillus from the alvine discharges of typhoid patients.

When the typhoid bacillus and the colon bacillus are planted together, in the same liquid medium, the first-mentioned bacillus, even when in excess at the outset of the experiment, soon disappears and the Bacillus coli communis remains in full possession. According to Wathelet (1895) the colon bacillus will grow in bouillon which has served as a culture medium for the typhoid bacillus, or on the surface of an agar plate from which a typhoid culture has been removed; but the typhoid bacillus fails to develop in culture media which have served for the development of the colon bacillus.

The various diagnostic tests which have been proposed, and the extensive literature of the subject, show that the recognition of the typhoid bacillus in water, fæces, etc., is attended with serious difficulties. This is chiefly due to the fact that bacilli have been obtained from various sources which resemble more or less closely the typical typhoid bacillus as obtained from the spleen of a typhoid patient (or cadaver) and the "colon bacillus" as found in the alimentary canal of healthy men and animals; and also from the fact that the bacillus, as obtained from typhoid cases, varies to some extent in its biological characters, and that varieties may be produced in the bacillus as obtained, from a single colony, by special modes of cultivation. From a consideration of these facts certain authors have been led to the conclusion that Bacillus typhi abdominalis and Bacillus coli communis are simply varieties of the same species. This view, however, is not generally accepted, and the characters which serve to differentiate the two bacilli are sufficiently well defined when typical cultures are compared. These characters, briefly stated, are: The invisible growth of the typhoid bacillus on potato; its failure to give the indol reaction; its failure to coagulate milk, or to produce a change of color in litmus milk; its failure to produce gas in culture media containing glucose or lactose; its failure to grow in formalin bouillon (1:7,000); and its active motility. Whether the closely related bacilli which present some of the characters above indicated, without corresponding in all particulars with typical cultures of the typhoid bacillus, are varieties of this bacillus, which under favorable circumstances could give rise to typhoid infection, has not been definitely determined, but appears to be quite probable. It may be that such varieties are developed when the typhoid bacillus in fæces finds its way into surface waters, under conditions which are favorable for its continued development as a saprophyte. On the other hand, it may be that one or more of the saprophytic bacilli, which are found in water and which closely resemble the typhoid bacillus, may give rise to the infectious disease which we know as typhoid fever when introduced into the alimentary canal of a particularly susceptible individual, and that the special conditions attending its development as a parasite give rise to certain modifications in its biological characters of a more or less permanent kind.

Frankland (1895), as a result of extended experiments, has arrived at the conclusion that when the typhoid bacillus is cultivated for a long time in media which are more and more largely diluted with water, it acquires an increased ability to survive in river water.

A predisposition to typhoid infection is established by various depressing agencies, such as inanition, overwork, mental worry, insanitary surroundings, etc. And there is considerable evidence in support of the supposition that exposure to the offensive gases given off from ill-ventilated sewers constitutes a predisposition to the disease.

Experiments made by Alessi (1894), in the Hygienic Institute of the University of Rome, give support to this view. The experiments were made upon rats, guinea-pigs, and rabbits. The rats were confined in a close cage with perforated bottom, which was placed over the opening of a privy; the guinea-pigs and rabbits in similar cages having a receptacle below in which their own excreta was allowed to accumulate. The animals which breathed an atmosphere vitiated in this way lost, after a time, their usual activity and became emaciated, although they continued to eat greedily. When these animals were inoculated with a small quantity of a culture of the typhoid bacillus (0.25 to 0.5 cubic centimetre) they died within from twelve to thirty-six hours. The same amount of the typhoid culture injected into control animals produced no injurious effect. In the animals which succumbed to typhoid infection there was found a hemorrhagic enteritis, increase in volume of Peyer's glands and of the spleen, and typhoid bacilli in the blood, liver, and spleen. The characteristic appearances of typhoid infection were more pronounced in the rabbits and guinea-pigs than in rats. Similar experiments with Bacillus coli communis gave similar results. The time required to induce this predisposition for typhoid infection was from five to seventy-two days for the rats, seven to fifty-eight for the guinea-pigs, and three to eighteen for the rabbits. Alessi found that the susceptibility to infection diminished after a certain time, and suggests that in a similar way man may become habituated to breathing an atmosphere containing sewer gases.

Pus-Production by Typhoid Bacilli.—The literature relating to the typhoid bacillus includes many observations as to its presence in accumulations of pus in various parts of the body—often in a pure culture. It has been found in a considerable number of cases of periostitis secondary to typhoid fever, in purulent synovitis, and in abscesses in various parts of the body.

Dmochowski and Janowski (1895), as the result of a review of the literature and a painstaking experimental research, arrive at the conclusion that even in abscesses, occurring in typhoid fever cases, in which only the pus cocci are found, it is probable that the typhoid bacillus originated the process resulting in abscess formation. They assert that the typhoid bacillus dies out in a comparatively short time in abscesses which are directly due to its presence, and that often it may be found in the abscess walls when its presence can no longer be demonstrated in the purulent contents of the abscess cavity.

PLATE V.

PATHOGENIC BACTERIA.

Fig. 1.—Bacillus anthracis from cellular tissue of inoculated mouse. Stained with gentian violet. × 1,000. Photomicrograph by Fränkel and Pfeiffer.

Fig. 2.—Bacillus anthracis in section of liver of inoculated rabbit. Stained with Bismarck brown. × 250. Photomicrograph by Sternberg.

Fig. 3.—Micrococcus gonorrhœæ in gonorrhœal pus. Stained with gentian violet. × 1,000. Photomicrograph by gaslight. (Sternberg.)

Fig. 4.—Anthrax spores from a bouillon culture. Double-stained preparation—with carbol-fuchsin and methylene blue. × 1,000. Photomicrograph by Fränkel and Pfeiffer.

Fig. 5.—Spirillum choleræ Asiaticæ from a culture upon starched linen at end of twenty-four hours. Stained with fuchsin. × 1,000. Photomicrograph by Fränkel and Pfeiffer.

Fig. 6.—Bacillus diphtheriæ from colony upon an agar plate, twenty-four hours old. Stained with Löffler's solution of methylene blue. × 1,000. Photomicrograph by Fränkel and Pfeiffer.

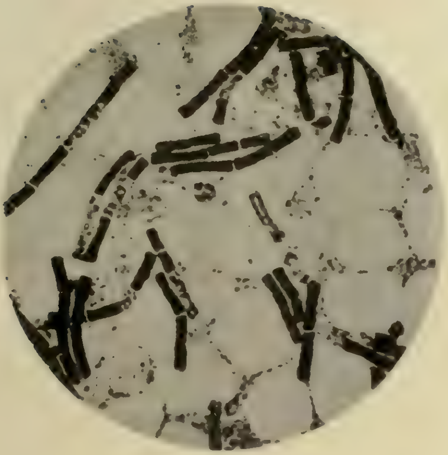

Fig. 1.

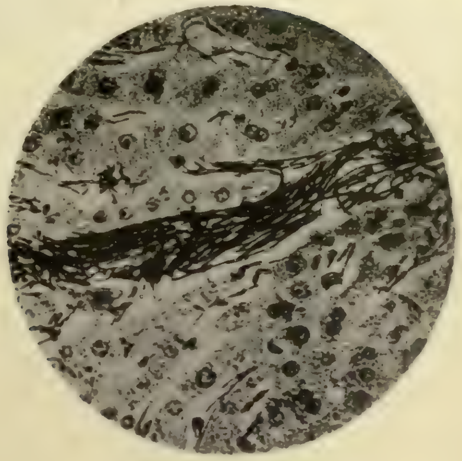

Fig. 2.

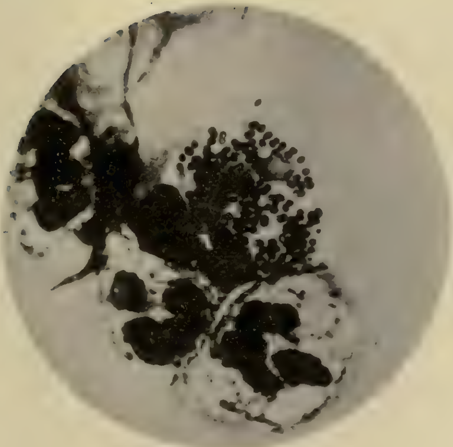

Fig. 3.

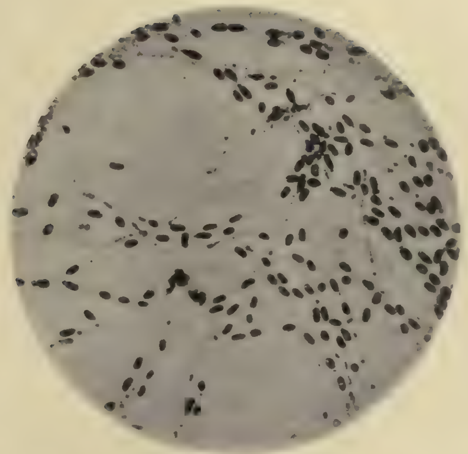

Fig. 4.

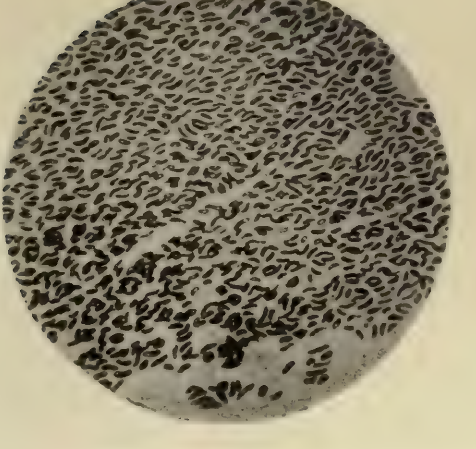

Fig. 5.

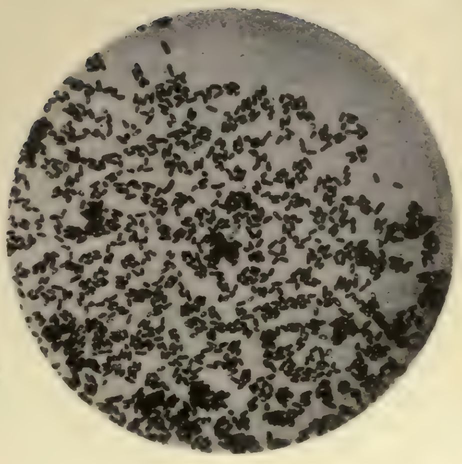

Fig. 6.

X.

BACTERIA IN DIPHTHERIA.

DIPHTHERIA is generally recognized by physicians as a specific infectious disease, and, owing to its wide prevalence and fatal character, a precise knowledge of its etiology is of the greatest importance. Until, as a result of recent researches, this was determined, pathologists were in doubt as to whether diphtheria should be considered as primarily a local infection, or whether the local manifestations were secondary to a general systemic infection. But this question appears now to be definitely settled in favor of the former view. We have to-day a very precise knowledge of the specific infecting agent, and have evidence that it produces during its growth a very potent toxic substance, the absorption of which from the seat of local infection accounts in a satisfactory manner for the general symptoms of the disease, which are due to toxæmia and not to an invasion of the blood and tissues by the pathogenic microörganism producing it.

Numerous researches by competent bacteriologists have failed to demonstrate the presence of bacteria in the blood of patients suffering from diphtheria, but a variety of microörganisms have been obtained in cultures from diphtheritic pseudo-membranes, and may be demonstrated by the microscopical examination of stained preparations. Among these are the well-known pus organisms, and especially the Streptococcus pyogenes, which appears to be very commonly present, and is perhaps the active agent in the production of certain forms of pseudo-diphtheria. But the malignant, specific diphtheria, so well known in this country and in Europe, has been demonstrated by the recent researches of bacteriologists to be due to a bacillus first recognized by Klebs in stained preparations of diphtheritic false membranes (1883), and cultivated and described by Löffler in 1884. In his first publication Löffler did not claim to have fully demonstrated the etiological relation of this bacillus, but this appears to be fully established by subsequent researches.

In his first research Löffler studied twenty-five cases, and in the greater number of them found in stained preparations the bacillus previously described by Klebs. From six of these cases he obtained it in pure cultures, and by inoculations in pigeons, chickens, rabbits, and guinea-pigs proved that it gave rise to a diphtheritic inflammation when inoculated into the mucous membrane of the trachea, conjunctiva, pharynx, or vagina. In a second communication Löffler reported his success in finding the same bacillus in ten additional cases, and also that he had isolated from the same source a non-pathogenic bacillus which resembled it very closely. This pseudo-diphtheria bacillus has since been found by other bacteriologists (Von Hoffmann, Roux and Yersin), and it is uncertain whether it is to be considered a distinct species, or a non-pathogenic variety of the diphtheria bacillus as maintained by Roux and Yersin. But its occasional presence does not invalidate the very positive experimental evidence relating to the specific pathogenic power of the true diphtheria bacillus.

Löffler, in 1890, reviewed the evidence upon which this bacillus is now generally conceded by bacteriologists to be the specific infectious agent in true diphtheria. The following are the principal points in the demonstration:

FIRST.—*It is found in all undoubted cases of diphtheria.* In support of this we have the results of researches made by Löffler, Wyssokowitsch, D'Espine, Von Hoffmann, Ortmann, Roux and Yersin, Kolisko and Paltauf, Zarinko and Sörensen, who in nearly every case have demonstrated without difficulty the presence of this bacillus. On the other hand, Prudden failed to find it in a series of twenty-four cases studied by him; but his own account of these cases indicates that they were not cases of true diphtheria. He says in a subsequent communication:

> "In view of the doubt existing among practitioners as to whether all forms of pseudo-membranous inflammation should be called diphtheria or not, and with the purpose of making a wholly objective study, the writer distinctly stated at the outset of that paper that all the fatal cases of extensive pseudo-membranous laryngitis, as well as pharyngitis, should in his study be considered as cases of diphtheria. This left the question as to the propriety of establishing separate groups of pseudo-membranous inflammation open and free from bias. It was distinctly stated, however, that sixteen out of the twenty-four cases occurred in a large asylum, in which measles and scarlet fever were prevalent during the period in which these studies were under way. Five other cases in another asylum were exposed to similar conditions."

In a subsequent series of "twelve cases of fatal pseudo-membranous inflammation occurring in two children's asylums, in which for many months there had been no scarlatina and no measles, and in which there was no complicating suppurative inflammation and no erysipelas," Prudden (1890) obtained Löffler's bacillus in cultures from eleven, and he says:

> "We are now, it would seem, justified, as it did not appear to the writer

that we were two years ago, owing to the large number of important researches which have been made in the interim, in saying that the name diphtheria, or at least primary diphtheria, should be applied, and exclusively applied, to that acute infectious disease, usually associated with a pseudomembranous inflammation of the mucous membranes, which is primarily caused by the bacillus called Bacillus diphtheriæ of Löffler."

With reference to the question as to how long after convalescence is established the diphtheria bacillus may be present in the throat of an infected person, Löffler has made the following research (1890). In a typical case a bacteriological examination was made daily from the commencement until fourteen days after its termination. Fever disappeared on the fifth day, and the exudation had all disappeared on the sixteenth day. Up to this time the bacillus was daily obtained in cultures, and subsequently nearly every day up to the twenty-fifth—that is, for three weeks after the febrile symptoms had disappeared. Roux and Yersin have also obtained the bacillus in cultures from mucus scraped from the throats of convalescents several days after the disappearance of all evidence of the disease.

SECOND. *The Klebs-Löffler bacillus is found only in diphtheria.*—In his earlier researches Löffler obtained the bacillus in a single instance from the mouth of a healthy child, and this fact led him to hesitate in announcing it as his conviction that it was the true cause of diphtheria. But in extended researches made subsequently he has not again succeeded in finding it, except in association with diphtheria, and admits now that he may have been mistaken as to the identity of the bacillus found. This seems not improbable in view of the fact that very similar bacilli have been found by various bacteriologists. Thus Von Hoffmann obtained a very similar but non-pathogenic bacillus from the mucus of chronic nasal catarrh and from healthy mucous membranes; Babes from cases of trachoma, Neisser from ulcers, Zarinko from the surface of various mucous membranes. But all of these were shown to present certain differences in their biological characters by which they could be differentiated from the true diphtheria bacillus.

Welch and Abbott in their comparative studies did not find the Löffler bacillus, "or any bacillus that an experienced bacteriologist would be likely to confound with it." They examined mucus from the throats of healthy children, from those suffering from simple inflammation of the tonsils and pharynx, and from four cases of so-called follicular tonsillitis. As a result of their investigations they agree with Löffler, and with Roux and Yersin, as to "the great practical value, for diagnostic purposes, of a bacteriological examination of cover-glass specimens and by cultures" of cases in which there is any doubt of the true character of the disease. They say further:

> "The only species of bacteria which we have found constantly in the cases of diphtheria has been the Löffler bacillus. Two other species have been present in many cases, viz., the well-known streptococcus, which grows in much smaller colonies and less rapidly than the Löffler bacillus, and a short, oval, often slightly pointed bacillus, growing in long chains running parallel to each other. There are often marked irregularities in shape and especially in size of this bacillus, even of individuals in the same chain. The colonies of this bacillus are grayish-white, moist, larger than those of the streptococcus, but smaller than those of the Löffler bacillus."

THIRD. *As shown by Löffler's earlier researches, pure cultures of this bacillus induce characteristic diphtheritic inflammation* when inoculated into the mucous membranes of certain lower animals. Roux and Yersin have also shown that local paralysis is likely to occur in inoculated animals, as is the case in diphtheria in man. In speaking of their inoculations into the trachea in rabbits these investigators say :

> "The affection which is thus induced in the rabbit resembles croup in man. The difficulty which the animal experiences in breathing; the noise made by the air in passing through the obstructed trachea· the aspect of the trachea, which is congested and covered with false membranes; the œdematous swelling of the tissues and glands of the neck, make the resemblance absolutely remarkable."

Welch and Abbott give the following account of the results of inoculations into the trachea in kittens :

> "A half-grown kitten is inoculated into the trachea with one platinum loop from a pure culture of the Löffler bacillus on glycerin-agar, eleven days old, derived from Case IV. For the inoculation a small median incision was made over the trachea, in which a hole just large enough to admit the platinum loop was made. The culture was rubbed over the mucosa of the trachea for an extent about three centimetres in length, and in this process sufficient force was used to abrade the mucous membrane. On the day following the inoculation no special alteration in the animal was observed, but on the morning of the second day it was found very weak. In the course of this day it became so weak as to lie completely motionless, apparently unconscious, with very feeble, shallow respiration; several times it was thought to be dead, but on careful examination proved still to be breathing feebly. It was found dead on the morning of the third day. At the autopsy the wound was found gaping and covered with a grayish, adherent, necrotic, distinctly diphtheritic layer. For a considerable distance around the wound the subcutaneous tissues were very œdematous, the œdema extending from the lower jaw down over the sternum, and to the sides of the neck, and along the anterior extremities. The lymphatic glands at the angle of the jaw were markedly swollen and reddened. The mucous membrane of the trachea, beginning at the larynx and extending down for six centimetres, was covered with a tolerably firm, grayish-white, loosely attached pseudo-membrane, in all respects identical with the croupous membranes observed in the same situation in cases of human diphtheria."

BACILLUS DIPHTHERIÆ.

First observed by Klebs (1883) in diphtheritic false membranes. Isolated in pure cultures and pathogenic power demonstrated by Löffler (1884).

Found in diphtheritic pseudo-membranes, and especially in the deeper portions, intermingled with numerous cellular elements; while the superficial layers of the membrane commonly contain but few cells or bacilli, or are invaded by other species, especially by Streptococcus pyogenes. The bacilli are not found in the affected mucous membrane, or in sections from the internal organs in fatal cases of this disease.

Morphology.—Rods, straight or slightly curved, with rounded ends, having a diameter of 0.5 to 0.8 μ, and from 2 to 3 μ in length. Irregular forms are very common, and, indeed, are characteristic of this bacillus. In the same culture, and especially in an unfavorable culture medium, very great differences in form and dimensions may be observed ; one or both ends may appear swollen, or the central portion may be notably thicker than the extremities, or the rod may be made up of irregular spherical or oval segments. Multiplication occurs by fission only, and the bacilli do not grow out into filaments.

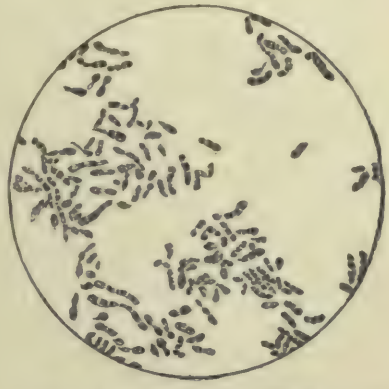

FIG. 112. — Bacillus diphtheriæ, from a culture upon blood serum. From a photomicrograph. × 1,000. (Fränkel and Pfeiffer.)

In unstained preparations certain portions of the rod, and especially the extremities, are observed to be more highly refractive than the remaining portion ; and in stained preparations these portions are seen to be most deeply colored. The diphtheria bacillus may be *stained* by the use of Löffler's alkaline solution of methylene blue, but is not so readily stained with some of the other aniline colors commonly employed. It stains also by Gram's method. For the demonstration of the bacillus in sections of diphtheritic membrane "nothing can surpass in brilliancy and sharp differentiation sections stained doubly by the modified Weigert's fibrin stain and. picro-carmine" (Welch and Abbott).

Biological Characters.—The diphtheria bacillus is *aërobic, non-motile*, and *non-liquefying;* it does not form spores. It grows most freely in the presence of oxygen, but is also a *facultative anaërobic.*

Development occurs in various culture media at a temperature of from 20° to 42° C., the most favorable temperature being about 35° C.

It grows readily in *nutrient gelatin* having a slightly alkaline reaction, in nutrient agar, glycerin-agar, or in alkaline bouillon, but the most favorable medium appears to be that first recommended by Löffler—viz., a mixture of three parts of blood serum with one part of bouillon, containing one per cent of peptone, one per cent of grape sugar, and 0.5 per cent of sodium chloride. This mixture is sterilized and solidified at a low temperature, as is usual with blood serum. Upon this the development is so rapid in the incubating oven that, at the end of twenty-four hours, the large, round, elevated colonies, of a grayish-white color and moist appearance, may be easily recognized, while other associated bacteria will, as a rule, not yet have developed colonies large enough to interfere with the recognition of these.

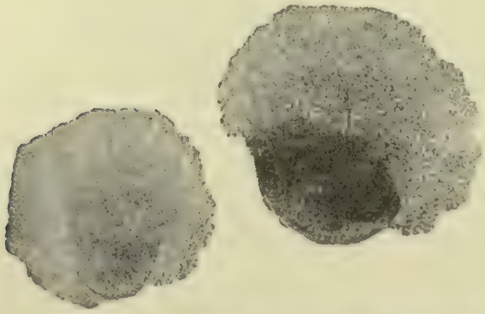

FIG. 113.—Colonies of Bacillus diphtheriæ in nutrient agar, end of twenty-four hours. × 10. (Fränkel and Pfeiffer.)

Upon *nutrient agar plates* the deep-lying colonies, when magnified about eighty diameters, appear as round or oval, coarsely granular discs, with rather ill-defined margins, or, when several colonies are in juxtaposition, as figures of irregular form. The superficial colonies are grayish-yellow in color, have an irregular, not well-defined outline and a rough, almost reticulated surface. The growth upon glycerin-agar is very similar. The first inoculations in a plain nutrient agar tube often give a comparatively feeble growth, which becomes more abundant in subsequent inoculations in the same medium. In stick cultures in glycerin—or plain—agar, growth occurs to the bottom of the line of inoculation, and also upon the surface, but is not at all characteristic. The same may be said with reference to cultures in nutrient gelatin. Plate cultures in this medium containing fifteen per cent of gelatin, at 24° C., give rather small colonies, which are white by reflected light and under the microscope are seen as yellowish-brown, opaque discs, having a more or less irregular outline and a granular structure. In alkaline *bouillon* the growth is sometimes in the form of small, whitish masses along the sides and bottom of the tube, but at others a diffusely clouded growth occurs in this medium; after standing for some time in the incubating oven a thin, white pellicle may form upon the surface of the bouillon. The reaction of the bouillon becomes at first acid, but later it has an alkaline reaction (Welch). With reference to the growth on *potato*, authors have differed, probably because the growth is scarcely visible; upon this point we quote from Welch and Abbott:

> "Our experience has been that the Bacillus diphtheriæ grows on ordinary steamed potato without any preliminary treatment, but that the growth is usually entirely invisible or is indicated by a dry, thin glaze after several days. Doubtless the invisible character of the growth has led most observers into the error of supposing that no growth existed, whereas the microscopical examination reveals a tolerably abundant growth, which on the first potato is often feebler than on succeeding ones. Irregular forms are particularly numerous in potato cultures, and in general the rods are thicker than on other media. In twenty-four hours, at a temperature of 35° C., microscopical examination shows distinct growth. We have cultivated the bacillus for many generations on potato."

Milk is a favorable medium for the growth of this bacillus, and, as it grows at a comparatively low temperature (20° C.), it is evident that this fluid may become a medium for conveying the bacillus from an infected source to the throats of previously healthy children.

Cultures of the diphtheria bacillus may retain their vitality for several months, and when dried upon silk threads for several weeks colonies are still developed in a suitable medium—in the room from three to four weeks, in an exsiccator five to ten, and in one instance fourteen weeks. In dried diphtheritic membrane, preserved in small fragments, the bacillus retained its vitality for nine weeks, and in larger fragments for twelve to fourteen weeks.

The thermal death-point, as determined by Welch and Abbott, is 58° C., the time of exposure being ten minutes. Löffler had previously found that it did not survive exposure for half an hour to 60° C. With reference to the action of germicidal and antiseptic agents, we refer to the sections in Part Second relating to this subject.

Pathogenesis.—In view of the evidence heretofore recorded, it may be considered as demonstrated that this bacillus gives rise to the morbid phenomena which characterize the fatal disease in man known as diphtheria.

We have already referred to the effects of inoculations into the trachea in rabbits and cats, which give rise to a characteristic diphtheritic inflammation, with general toxæmia and death from the absorption of soluble toxic products formed at the seat of local infection. This inference as to the cause of death seems justified by the fact that the pathogenic bacillus does not invade the blood and tissues, and is supported by additional experimental evidence (see pages 309–317).

PSEUDO-DIPHTHERITIC BACILLUS.

Löffler, Von Hoffmann, and others have reported finding bacilli which closely resemble the Bacillus diphtheriæ, but which differ from it chiefly in being non-pathogenic. The following account we take from a paper upon the subject by Roux and Yersin (troisième mémoire, 1890).

Found by Roux and Yersin in mucus from the pharynx and tonsils of children—from forty-five children in Paris hospitals, suffering from various affections, not diphtheritic, fifteen times; from fifty-nine healthy children in a village school on the seaboard, twenty-six times. Of six children with a simple angina but two furnished cultures of this bacillus, while it was obtained in five out of seven cases of measles.

Its characters are given as follows:

"The colonies of the pseudo-diphtheritic bacillus, cultivated upon blood serum, are identical with the true diphtheria bacillus At a temperature of 33° to 35° multiplication is rapid, and it continues at the ordinary temperature, although slowly. Under the microscope the appearance of the bacillus which forms these colonies is the same as that of Bacillus diphtheriæ. It stains readily with Löffler's solution of methylene blue, and intensely by Gram's method. Sometimes it colors uniformly, at others it appears granular. It grows in alkaline bouillon, giving a deposit upon the walls of the vessel containing the culture, and in this medium often presents the inflated forms, pear-shaped, or club-shaped. It is destroyed in a liquid medium by a temperature of 58° C. maintained for ten minutes. All of these characters are common to the pseudo-diphtheritic bacillus and the true Bacillus diphtheriæ. As a difference between them we may note that the pseudo diphtheritic bacillus is often shorter in colonies grown upon blood serum; that its cultures in bouillon are more abundant; that they continue at a temperature of 20° to 22°, at which the true bacillus grows very slowly. When we make a comparison of cultures in bouillon they become acid and then alkaline, but the change occurs much sooner in the case of the pseudo-diphtheritic bacillus. Like the true bacillus, the pseudo diphtheritic grows in a vacuum, but less abundantly than the other.

"Inoculations into animals of cultures of this bacillus have never caused their death; but we may remark that in some experiments a notable œdema has been produced in guinea-pigs at the point of inoculation, while in others there has been no local lesion. The most marked œdema resulted from cultures obtained from cases of measles.

"Do the facts which we have reported explain the question which occupies us? Can we conclude that there is a relation between the two bacilli? On the one side, the presence of the pseudo diphtheritic bacillus in the mouths of healthy persons, and of those who have anginas manifestly not diphtheritic. seems to be opposed to the idea of a relationship between them. On the other hand, when we consider that the non-virulent bacillus is very rare in fatal diphtheria, that it is more abundant in benign diphtheria, that it becomes more common in severe cases as they progress towards recovery, and, finally, that they are more numerous in persons who have recently had diphtheria than in healthy persons, it is difficult to accept the idea that the two microbes are entirely distinct. The morphological differences which have been referred to are so slight that they prove nothing. The two micro-örganisms can only be distinguished by their action upon animals, but the difference of virulence does not at all correspond with the difference of origin. As regards the form and the aspect of cultures, the true and false diphtheria bacilli differ less than virulent anthrax differs from a very attenuated anthrax bacillus, which, however, originate from the same source. Besides, the sharp distinction which we make between the virulent and non-virulent bacilli is arbitrary; it depends upon the susceptibility of guinea-pigs. If we inoculate animals still more susceptible, there are pseudo diphtheritic bacilli which we must class as virulent; and if, on the contrary, we substitute rabbits for guinea pigs in our experiments, there are diphtheritic bacilli which we must call pseudo-diphtheritic. In our experiments we do not simply encounter bacilli which are very virulent and bacilli which are non-virulent; between these two extremes there are bacilli of every degree of virulence."

Abbott, in 1891, published the result of his researches with reference to the presence of the pseudo-diphtheritic bacillus in benign throat affections. He made a bacteriological study of fifty-three patients, nine of whom were suffering from acute pharyngitis, fourteen from acute follicular tonsillitis, eight from ordinary post-nasal catarrh, two from simple enlarged tonsils, fifteen from chronic pharyngitis, one from subacute laryngitis, one from chronic laryngitis, one from rhinitis, and two from an affection of the tonsils and pharynx. In forty-nine cases nothing of particular interest was observed. A variety of microörganisms were isolated, and of these the pyogenic micrococci were the most common.

In four cases microörganisms were found which resembled the Bacillus diphtheriæ of Löffler in their morphology and growth in culture media, but which proved not to be pathogenic. Abbott says: "The single point of distinction that can be made out between the organisms obtained from Cases I., III., and IV. and the true bacillus of diphtheria is in the absence of pathogenic properties from the former, whereas in addition to this point of distinction the organism from Case II. gives, as has been stated, a decided and distinct growth upon the surface of sterilized potato."

Recent authors are generally inclined to the opinion that bacilli which resemble the diphtheria bacilli in every respect except that they are non-pathogenic should be regarded as attenuated varieties of the diphtheria bacillus rather than as belonging to a distinct species—the so-called "pseudo-diphtheria" bacillus. However, there are bacilli which closely resemble the bacillus of diphtheria and yet may be differentiated from it otherwise than by the test upon susceptible animals. Neisser has given us a staining method which is especially useful in making this differential diagnosis. The culture of the bacillus to be tested is grown upon Löffler's blood-serum mixture. This is solidified at a temperature of 100° C., and grown in an incubator at a temperature between 34° and 36° C. The staining of a cover-glass preparation from such a culture is effected by the following method: Methylene blue, one gramme; alcohol (96°), two cubic centimetres; dissolve and add distilled water, nine hundred and fifty cubic centimetres, and acetic acid, fifty cubic centimetres. From one to three seconds only will be required to stain the cover-glass preparation with this solution; it should then be carefully washed in water and stained in a solution made by adding two grammes of vesuvin to one litre of boiling water. This solution is allowed to cool before using, and from three to five seconds will be sufficient time for the action of the stain, after which the cover glass is again washed and is then ready for examination. The diphtheria bacillus appears in such a preparation as faintly stained brown rods, in the interior of which one to three dark-blue granules may be seen. These are oval in form and are found at the extremities of the bacterial cells. Neisser and others who have made use of this method agree that bacilli which do not stain in this way are not diphtheria bacilli.

BACILLUS DIPHTHERIÆ COLUMBARUM.

Described by Löffler (1884), who obtained it from diphtheritic pseudo-membranes in the mouths of pigeons dead from an infectious form of diphtheria which prevails in some parts of Germany among these birds and among chickens.

Reddened patches first appear upon the mucous membrane of the mouth and fauces, and these are covered later with a rather thick, yellowish layer of fibrinous exudate. In pigeons the back part of the tongue, the fauces, and the corners of the mouth are especially affected; in chickens the tongue, the gums, the nares, the larynx, and the conjunctival mucous membrane. The disease is especially fatal among chickens, the young fowls and those of choice varieties being most susceptible. It is attended at the outset by fever, and usually proves fatal within two or three weeks, but may last for several months.

Morphology.—Short bacilli with rounded ends, usually associated in irregular masses, and resembling the bacilli of rabbit septicæmia (fowl cholera), but a little longer and not quite so broad. In sections from the liver they are seen in irregular groups in the interior of the vessels.

Biological Characters —An *aërobic*, *non-motile*, *non-liquefying* bacillus.

Grows in *nutrient gelatin* in the form of spherical, white colonies along the line of puncture, and upon the surface as a whitish layer. Under the microscope the colonies in gelatin plates have a yellowish-brown color and a slightly granular surface. Upon *blood serum* the growth consists of a semi-transparent, grayish-white layer. Upon *potato* a thin layer is formed having a grayish tint.

Pathogenesis.—Pigeons inoculated with a pure culture in the mucous membrane of the mouth are affected exactly as are those which acquire the disease naturally. Subcutaneous inoculations in pigeons give rise to an inflammation resulting in local necrotic changes. Pathogenic for rabbits and for mice. Subcutaneous injections in mice give rise to a fatal result in about five days. The bacillus is found in the blood and in the various organs, in the interior of the vessels, and sometimes in the interior of the leucocytes; they are especially numerous in the liver. The lungs are dotted with red spots, the spleen is greatly enlarged, and the liver has a marbled appearance from the presence of numerous irregular white masses scattered through the pale-red parenchyma of the organ. These white masses are seen, in sections, to consist of necrotic liver tissue, in the centre of which the bacilli are found in great numbers, in the interior of the vessels. This appearance is so characteristic that Löffler considers inoculations in mice to be the most reliable method of establishing the identity of the bacillus. Not pathogenic for chickens, guinea-pigs, rats, or dogs.

There seems to be some doubt whether the form of diphtheria which prevails among pigeons, and which Löffler has shown to be due to the bacillus above described, is identical with the diphtheria of chickens. Diphtheria in man has been supposed by some authors to be identical with that which prevails among fowls, and possibly this may be the case under certain circumstances. But the evidence seems to be convincing that there is an infectious diphtheria of fowls which is peculiar to them, and which, under ordinary circumstances, is not communicated to man.

BACILLUS DIPHTHERIÆ VITULORUM.

Described by Löffler (1884) and obtained by him from the pseudo-membranous exudation in the mouths of calves suffering from an infectious form of diphtheria. The disease is characterized by the appearance of yellow patches upon the mucous membrane of the cheeks, the gums, the tongue, and sometimes of the larynx and nares of infected animals. There is a yellowish discharge from the nose, an abundant flow of saliva, occasional attacks of coughing, and diarrhœa. Death may occur at the end of four or five days, but usually the animal survives for several weeks. Diphtheritic patches similar to those in the mouth are also found in the large intestine, and scattered abscesses in the lungs.

Löffler, in a series of seven cases examined, obtained from the deeper portions of the pseudo-membranous deposit a long bacillus which appears to be the cause of the disease.

Morphology.—Bacilli, five to six times as long as broad, usually united in long filaments. The diameter of the rods is about half that of the bacillus of malignant œdema.

Biological Characters.—Attempts to cultivate this bacillus in nutrient gelatin, blood serum from sheep, and various other media were unsuccessful. But when fragments of tissue containing the bacillus were placed in blood serum from the calf a whitish border, consisting of the long bacilli, was developed. These could not, however, be made to grow when transferred to fresh blood serum.

Pathogenesis.—Mice inoculated subcutaneously with the fresh diphtheritic exudation died in from seven to thirty days. The autopsy disclosed an extensive infiltration of the entire walls of the abdomen, which often penetrated the peritoneal cavity and enveloped the liver, the kidneys, and the intestine in a yellowish exudate. The bacillus was found in this exudate, and by inoculating a little of it into another animal of the same species a similar result was obtained. Not pathogenic for rabbits or guinea-pigs.

BACILLUS OF INTESTINAL DIPHTHERIA IN RABBITS.

Described by Ribbert (1887) and obtained by him from the organs of rabbits which succumbed to an affection characterized by a diphtheritic inflammation of the mucous membrane of the intestine. The autopsy revealed also swelling of the mesenteric glands and minute necrotic foci in the liver and spleen.

Morphology.—Bacilli with slightly rounded ends, from three to four μ long and 1 to 1.4 μ in diameter; often united in pairs or in filaments containing several elements.

Stains with the aniline colors, but not so readily in sections as some other microörganisms. Ribbert recommends staining with aniline-water-fuchsin solution, washing in water, then placing the sections in methylene blue solution, and decolorizing in alcohol. Does not stain by Gram's method.

Biological Characters.—An *aërobic, non-liquefying* (non-motile ?) bacillus. Upon *gelatin plates* semi-transparent, grayish colonies are formed, which later have a brownish color; the surface of these is finely granular and of a pearly lustre. In stick cultures in nutrient gelatin the growth along the line of puncture is very scanty. On *potato* a flat, whitish layer is formed, which extends slowly over the surface. Grows best at a temperature of 30° to 35° C.

Pathogenesis.—Pure cultures injected into the peritoneal cavity or subcutaneously in rabbits caused the death of these animals in from three to fourteen days, according to the quantity injected. At the autopsy necrotic foci are found in the liver and spleen, and the mesenteric glands are enlarged, but the intestine presents a healthy appearance. But when cultures are introduced into the alimentary canal the characteristic diphtheritic inflammation of the mucous membrane of the intestine is induced. This result was obtained both by direct injection into the lumen of the intestine and by injecting cultures into the mouth.

Additional Notes upon Diphtheria and the Diphtheria Bacillus.—C. Fränkel (1895) reports that he has repeatedly observed branching forms of the diphtheria bacillus in cultures upon Löffler's blood-serum medium, and that these branching forms are seen more constantly and in greater numbers in cultures made upon the surface of hard-cooked albumen from hen's eggs.

The continued presence of virulent diphtheria bacilli in the fauces of patients who have recovered from the disease, either after the use of the antitoxin or under other treatment, has been demonstrated by several bacteriologists. Silverschmidt (1895), in forty-five cases treated by Behring's antitoxic serum, found that the number of bacilli usually diminished some days after the treatment was commenced, but that in cases in which complete recovery had taken place not infrequently virulent bacilli could be obtained many days (in one case thirty-one days) after convalescence was established.

Escherich (1893) opposes the view that the pseudo-diphtheria bacillus is simply a non-virulent variety of the diphtheria bacillus. He found this pseudo-diphtheria bacillus in the throats of thirteen out of three hundred and twenty individuals examined. According to him there is no evidence that this completely non-virulent pseudo-diphtheria bacillus ever acquires pathogenic virulence, while attenuated varieties of the true diphtheria bacillus readily recover their power to produce the toxic products upon which virulence depends.

Sevestre (1895), as a result of researches made by himself and several other bacteriologists who have made similar investigations, arrives at the conclusion that:

"First. In a certain number of cases the bacillus of Löffler disappears about the same time as the false membranes; or it may persist for some time, but ceases to be virulent—in this case it seems to have undergone modifications and presents the form of short bacilli. . . .

"Second. In another series of cases, less numerous but nevertheless considerable, the bacillus persists in a virulent condition for a longer or shorter time after the apparent cure of the malady. . . .

"Third. The observations collected up to the present time do not enable us to fix precisely the limits of persistence, but it is not far out of the way if we place it at several weeks to a month for the throat. In the nasal fossæ the bacillus often persists for a still longer time, and its presence commonly coincides with a more or less abundant discharge from the nose."

Park and Beebe (1894), in an extended research made for the purpose of determining the persistence of the diphtheria bacillus in the throats of convalescents (2,566 cultures made), found that in 304 out of 605 consecutive cases the bacillus disappeared within 3 days after the disappearance of the exudate; in 176 cases it persisted for 7 days; in 64 cases for 12 days; in 36 cases for 15 days; in 12 cases for 3 weeks; in 4 cases for 4 weeks; in 2 cases for 9 weeks. Park and Beebe arrive at the following conclusion with reference to pseudo-diphtheria bacilli:

"The name pseudo-diphtheria bacillus should be regarded as applying to those bacilli found in the throat which, though resembling the diphtheria bacilli in many respects, yet differ in others equally important. These bacilli are rather short, and more uniform in size and shape than the typical Löffler bacillus. They stain equally throughout with the alkaline methyl-blue solution, and produce alkali in their growths in bouillon. They are found in about one per cent of the healthy throats in New York City, and seem to have no connection with diphtheria. They are never virulent."

Park (1894) has shown that virulent diphtheria bacilli are frequently found in the throats of persons who have been associated with diphtheria patients, although no manifestations of the disease were visible. It is therefore apparent that infection requires not only the presence of virulent bacilli, but also of a predisposition to the disease. This corresponds with the facts relating to other infectious diseases—*e.g.*, tuberculosis, typhoid fever—and among the probable predisposing causes we may mention "sewer-gas poisoning," catarrhal inflammations of the mucous membranes most commonly involved, inanition, "crowd poisoning," and depressing agencies generally.

Bacteriologists have given much attention to the question of *mixed infection* in diphtheria. Funck (1894) accepts the generally received view that mixed infections with the diphtheria bacillus and Streptococcus pyogenes are more serious than an uncomplicated diphtheria, and in an experimental research has attempted to determine whether this is due to an increased production of the diphtheria bacillus or to the presence of the streptococcus. His experiments on guinea-pigs showed that when infected with streptococci these animals did not prove to be more sensitive to the action of the diphtheria poison (without living bacilli), and he concludes that the unfavorable influence of the streptococcus in mixed infections is due to increased pathogenic activity on the part of the diphtheria bacillus. Bernheim (1894) found, in his experiments on guinea-pigs, that they succumbed more rapidly to diphtheria infection when they previously or simultaneously received an injection of a streptococcus culture—filtered or unfiltered.

Results of Treatment with the Antitoxin.—While questions relating to therapeutics are not considered in this manual, a brief note upon the results of treatment by the serum of immunized animals may not be out of place. A collective investigation (1895) undertaken by the *Deutsche medicinische Wochenschrift* gave the following results: The number of cases collected was 10,312; all of these occurred between the 1st of October, 1894, and the 1st of April, 1895; 5,883 of these cases were treated with the antitoxin and 4,479 without it. In the first group the mortality was 9.6 per cent, and in the second group 14.7 per cent. Two thousand five hundred and fifty six children treated with the antitoxin were between two and ten years of age; among these the mortality was 4 per cent, while among children of the same age not treated with the antitoxin the mortality was 15.2 per cent. Six hundred and ninety-six patients above ten years of age were treated with a mortality of 1 per cent.

Monod (1895), at a meeting of the Paris Academy of Medicine, presented the following statistics demonstrating the influence upon the mortality from diphtheria in France exerted by the antitoxin since its employment from November, 1894. The following figures represent the number of deaths from diphtheria during the first six months in eight years in 108 French cities having a population of more than 20,000:

	1888-94. Average.	1895. Average.
January	469	205
February	466	187
March	499	155
April	442	160
May	417	113
June	333	84
	2,656	904

It will be seen from the above statement that during the first six months in the year 1895 after the introduction of the antitoxin treatment, the number of deaths from diphtheria in the 108 French cities referred to was 1,552 less than the average for the preceding ten years, and we are justified in concluding that a considerable proportion of this saving at least is due to this method of treatment.

XI.

BACILLUS OF INFLUENZA.

Discovered by Pfeiffer (1892) in the purulent bronchial secretion, and by Canon in the blood of patients suffering from epidemic influenza. Pfeiffer found the bacillus in thirty-one cases examined by him, and in uncomplicated cases it was present in the purulent bronchial secretion in immense numbers and in a pure culture. Canon, whose independent observations were published at the same time, examined the blood of twenty influenza patients in stained preparations, and found the same bacillus in nearly all of them. His method of demonstrating it is as follows:

The blood is spread upon clean glass covers in the usual way. After the preparations are thoroughly dry they are placed in absolute alcohol for five minutes. They are then transferred to the following staining solution (Czenzynke's): concentrated aqueous solution of methylene blue, forty grammes; one-half-per-cent solution of eosin (dissolved in seventy-per-cent alcohol), twenty grammes; distilled water, forty grammes. The cover glasses immersed in this staining solution are placed in an incubating oven at 37° C. for from three to six hours, after which they are washed with water, dried, and mounted in balsam. In successful preparations the red blood corpuscles are stained red by the eosin, and the leucocytes blue. The bacillus is seen in these as a short rod, often resembling a diplococcus. It is sometimes seen in large numbers, but usually only a few rods are seen after a long search—four to twenty in a single preparation. In six cases it was found in numerous aggregations containing from five to fifty bacilli each. In these cases the blood was drawn during a fall of temperature or shortly after.

Morphology.—Very small bacilli, having about the same diameter as the bacillus of mouse septicæmia, but only half as long. Solitary or united in chains of three or four elements.

Stains with difficulty with the basic aniline dyes—best with dilute Ziehl's solution, or Löffler's methylene blue solution, with heat. The two ends of the bacilli are most deeply stained, causing them to resemble diplococci. Pfeiffer says: "I am inclined to believe that some of the earlier observers also saw the bacilli described by me, but that, misled by their peculiar behavior with regard to staining agents, they described them as diplococci or streptococci." Do not stain by Gram's method.

Biological Characters.—An *aërobic, non-motile* bacillus. Does not grow in nutrient gelatin at the room temperature. Spore formation not observed. Upon the surface of *glycerin-agar* in the incubating oven very small, transparent, drop-like colonies are developed at the end of twenty-four hours. These can only be recognized by the aid of a lens. "A remarkable point about them is that the colonies always remain separate from each other, and do not, as all other species known to me do, join together and form a continuous row. This feature is so characteristic that the influenza bacilli can be thereby with certainty distinguished from other bacteria" (Kitasato). On 1.5 per cent sugar-agar the colonies appear as extremely small droplets, clear as water, often only recognizable with a lens (Pfeiffer).

In *bouillon* a scanty development occurs, and at the end of twenty-four hours small, white particles are seen upon the surface, which subsequently sink to the bottom, forming a white, woolly deposit, while the bouillon above remains transparent. This bacillus does not grow at temperatures below 28° C.

Canon has obtained colonies, resembling those described by Kitasato, in cultures from the blood of influenza patients. His cultures were made upon glycerin-agar in Petri's dishes. Ten or twelve drops of blood from a puncture made in the finger of the patient, after sterilization of the surface, were allowed to fall upon the agar medium, and this was placed in the incubating oven. As the number of bacilli in the blood is small, a considerable quantity is used. The colonies are visible at the end of twenty-four to forty-eight hours.

The influenza bacillus is quickly destroyed by desiccation; a pure culture diluted with water and dried is destroyed with certainty in twenty hours; in dried sputum the vitality is retained somewhat longer, but no growth occurs after forty hours. The thermal death-point is 60° C. with five minutes' exposure (Pfeiffer and Beck).

Pathogenesis.—Pfeiffer infers that this is the specific cause of influenza in man for the following reasons:

1. They were found in all uncomplicated cases of influenza examined, in the characteristic purulent bronchial secretion, often in absolutely pure cultures. They were frequently situated in the protoplasm of the pus corpuscles; in fatal cases they were found to have penetrated from the bronchial tubes into the peribronchitic tissue, and even to the surface of the pleura, where in two cases they were found in pure cultures in the purulent exudation.

2. They were only found in cases of influenza. Numerous control experiments proved their absence in ordinary bronchial catarrh, etc.

PLATE VI.

PATHOGENIC BACTERIA.

Fig. 1.—Bacillus of influenza in bronchial mucus. × 1,000. Photomicrograph by Fränkel.

Fig. 2.—Bacillus of influenza in bronchial mucus, after the termination of the febrile period. The bacilli are for the most part in pus cells. × 1,000. Photomicrograph by Fränkel.

Fig. 3.—Bacillus tetani from an agar culture. × 1,000. Photomicrograph by Fränkel and Pfeiffer.

Fig. 4.—Micrococcus pneumoniæ crouposæ in sputum of a patient with pneumonia. × 1,000. Stained by Gram's method. Photomicrograph by Fränkel and Pfeiffer.

Fig. 5.—Micrococcus pneumoniæ crouposæ in blood of rabbit. × 1,000. Photomicrograph made at the Army Medical Museum, Washington, by Gray.

Fig. 6.—Bacillus of hog cholera, showing flagella. Stained by Löffler's method. × 1,000. Photomicrograph made at the Army Medical Museum, Washington, by Gray.

3. The presence of the bacilli corresponded with the course of the disease, and they disappeared with the cessation of the purulent bronchial secretion.

In his preliminary report of his investigations Pfeiffer says :

"Numerous inoculation experiments were made on apes, rabbits, guinea-pigs, rats, pigeons, and mice. Only in apes and rabbits could positive results be obtained. The other species of animals showed themselves refractory to influenza."

Kruse (1894) reports that he found the bacillus of Pfeiffer in eighteen influenza patients examined by him in the hospital at Bonn. On the other hand, he failed to find it in a considerable number of patients suffering from other diseases of the respiratory passages. His evidence is the more valuable as he had previously (1890) reported his failure to find the bacillus in typical cases of influenza. He now ascribes his failure at that time to imperfect technique.

Huber (1893), Richter (1894), Borchardt (1894), and other competent bacteriologists, have also confirmed the results reported by Pfeiffer as regards the presence of this bacillus in the bronchial secretions of persons suffering from epidemic influenza, and as to its biological characters. Bujwid (1893) recognizes the bacillus of Pfeiffer as identical with a bacillus which he cultivated from the spleen of an influenza patient in 1890.

The researches of Weichselbaum, Kowalski, Friedrich, Kruse, Bouchard, and others have given a negative result as regards the presence of the influenza bacillus in the blood. They were not able to demonstrate its presence either in stained preparations or by culture methods. Pfeiffer, also, during the last epidemic, has made special researches upon this point and has never succeeded in finding the bacillus. Day after day, both in mild and severe cases, he placed from ten to twenty drops of blood from influenza patients on blood-agar—a most favorable medium—but his cultures always remained sterile.

In his experiments upon rabbits, Pfeiffer (1893) found that the intravenous injection of a small quantity of culture on blood-agar, twenty-four hours old, suspended in one cubic centimetre of bouillon, caused a characteristic pathogenic effect. The first symptoms were developed within one and a half to two hours after the injection. The animals became extremely feeble, lying flat upon the floor with their limbs extended, and suffered from extreme dyspnœa. The temperature mounted to 41° C. or above. At the end of five or six hours they were able to sit upon their haunches again, and in twenty-four hours had nearly recovered from all indications of ill-health. Larger doses caused the death of the inoculated animals. These results are due to toxic products present in the cultures, and Pfeiffer has never observed a septicæmic infection as a result of his inoculation experiments.

Pfeiffer has found in three cases of bronchopneumonia a pseudo-influenza bacillus which closely resembles the bacillus previously described by him as peculiar to that disease. This pseudo-influenza bacillus resembles the genuine one in its growth in culture media, but is larger and shows a decided inclination to grow out into long threads. By these morphological characters, which are said to be constant, it may, according to Pfeiffer, be readily distinguished.

XII.

BACILLI IN CHRONIC INFECTIOUS DISEASES.

In tuberculosis, leprosy, glanders, and syphilis we have a group of infectious diseases which present many points of resemblance. All run a chronic course; all may be communicated to susceptible animals by inoculation; in all, the lymphatic glands in the vicinity of the point of inoculation become enlarged, and new growths, consisting of various cellular elements of a low grade of vitality, are developed in the tissues which are the point of predilection for each; in all, these new growths show a tendency to degenerative changes, as a result of which abscesses, caseous masses, or open ulcers are formed.

In two of the diseases in this group—tuberculosis and glanders—the infectious agent has been obtained in pure cultures and its specific pathogenic power demonstrated by inoculations in susceptible animals; in one—leprosy—there is but little doubt that the bacillus constantly found in the new growths characteristic of the disease bears an etiological relation to it, although this has not been demonstrated, the bacillus not having as yet been cultivated in artificial media. The evidence with reference to the parasitic nature of the fourth disease mentioned as belonging to this group—syphilis—is still unsatisfactory, but there is every reason to believe that it will also eventually be proved to be due to a parasitic microörganism.

The announcement of the discovery of the tubercle bacillus was made by Koch, in March, 1882, at a meeting of the Physiological Society of Berlin. At the same time satisfactory experimental evidence was presented as to its etiological relation to tuberculosis in man and in the susceptible lower animals, and its principal biological characters were given.

Baumgarten independently demonstrated the presence of the tubercle bacillus in tuberculous tissues and published the fact soon after the appearance of Koch's first paper. The previous demonstration by Villemin (1865)—confirmed by Cohnheim (1877) and others—that tuberculosis might be induced in healthy animals by inoculations of tuberculous material, had paved the way for his discovery, and advanced pathologists were quite prepared to accept it. The more conservative have since been obliged to yield to the experimental evidence, which has received confirmation in all parts of the world. To-day it is generally recognized that tuberculosis is a specific infectious disease due to the tubercle bacillus.

As evidence of the thorough nature of Koch's personal researches in advance of his first public announcement, we give the following résumé of his investigations :

In nineteen cases of miliary tuberculosis the bacilli were found in the tubercular nodules in every instance ; also in twenty-nine cases of pulmonary phthisis, in the sputum, in fresh cheesy masses, and in the interior of recently formed cavities ; in tuberculous ulcers of the tongue, tuberculosis of the uterus, testicles, etc. ; in twenty-one cases of tuberculous—scrofulous—lymphatic glands ; in thirteen cases of tuberculous joints ; in ten cases of tubercular bone affections ; in four cases of lupus ; in seventeen cases of Perlsucht in cattle. His experimental inoculations were made upon two hundred and seventy-three guinea-pigs, one hundred and five rabbits, forty-four field mice, twenty-eight white mice, nineteen rats, thirteen cats, and upon dogs, pigeons, chickens, etc. Very extensive comparative researches were also made, which convinced him that the bacillus which he had been able to demonstrate in tuberculous sputum and tissues by a special mode of staining was not to be found in the sputa of healthy persons, or of those suffering from non-tubercular pulmonary affections, or in organs and tissues involved in morbid processes of a different nature.

BACILLUS TUBERCULOSIS.

Discovered by Koch (first public announcement of discovery March 24th, 1882). The bacilli are found in the sputum of persons suffering from pulmonary or laryngeal tuberculosis, either free or in the interior of pus cells ; in miliary tubercles and fresh caseous masses, in the lungs or elsewhere ; in recent tuberculous cavities in the lungs ; in tuberculous glands, joints, bones, and skin affections (lupus) ; in the lungs of cattle suffering from pulmonary tuberculosis—Perlsucht ; and in tubercular nodules generally in animals which are infected naturally or by experimental inoculations.

In the giant cells of tubercular growths they have a peculiar and characteristic position, being found, as a rule, upon the side of the cell opposite to the nuclei, which are crowded together in a crescentic arrangement at the opposite pole of the cell. Sometimes a single bacillus will be found in this position, or there may be several. Again, numerous bacilli may be found in giant cells in which the nuclei are distributed around the periphery. They are more numerous in tuberculous growths of recent origin, and often cannot be demonstrated, by microscopical examination, in caseous material from the centre of older nodules. But such material, when inoculated into susceptible animals, gives rise to tuberculosis, and the usual inference is that it contains spores of the tubercle bacillus.

Morphology.—The tubercle bacilli are rods with rounded ends, of from 1.5 to 3.5 μ in length, and are commonly slightly curved or bent at an angle; the diameter is about 0.2 μ. In stained preparations unstained portions are frequently seen, which are generally believed to be spores, but this is by no means certain. From two to six of these unstained spaces may often be seen in a single rod, and owing to this alternation of stained and unstained portions the bacilli may, under a low power, be mistaken for chains of micrococci The rods are usually solitary, but may be united in pairs, or in short chains containing three or four elements. In old cultures irregular forms may be observed, the rods being sometimes swollen at one extremity, or presenting the appearance of having a lateral bud-like projection—involution forms.

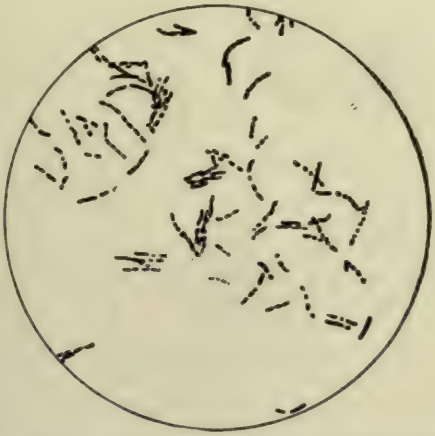

FIG. 114. — Bacillus tuberculosis. × 1,000. From a photomicrograph.

The *staining* characters of this bacillus are extremely important for its differentiation and recognition in preparations of sputum, etc. Unlike most microörganisms of the same class, it does not readily take up the aniline colors, and when stained it is not easily decolorized, even by the use of strong acids. The failure to observe it in tuberculous material, prior to Koch's discovery, was no doubt due to the fact that it does not stain in the usual aqueous solutions of the aniline dyes. Koch first recognized it in preparations placed in a staining fluid to which an alkali had been added—solution of methylene blue with caustic potash; but this method was not very satisfactory, and he promptly adopted the method devised by Ehrlich, which consists essentially in the use of a solution of an aniline color—fuchsin or methyl violet—in a saturated aqueous solution of aniline oil, and decolorization with a solution of a mineral acid—nitric acid one part to three parts of water.

The original method of Ehrlich gives very satisfactory results, but various modifications have since been proposed, some of which are advantageous. The carbol-fuchsin solution of Ziehl is now largely employed; it has the advantage of prompt action and of keeping well. The staining is effected more quickly if heat is applied. The tubercle bacilli stain by Gram's method, but this is not to be recommended for general use, owing to the fact that the protoplasm of the rods is frequently contracted into a series of spherical, stained bodies, which might easily be mistaken for micrococci.

The *examination of sputum* for the presence of the tubercle bacillus is recognized as a most important procedure for the early diagnosis of pulmonary tuberculosis. It is attended with no special difficulties, and every physician should be acquainted with the technique.

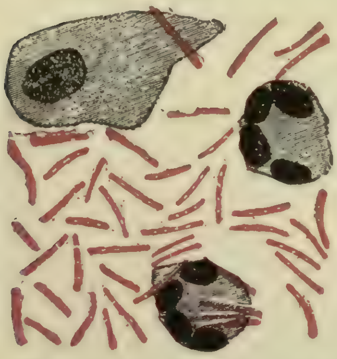

FIG. 115.—Bacillus tuberculosis in sputum. × 1,000. (Baumgarten.)

The patient should be directed to expectorate into a clean, wide-mouthed bottle or glass-covered jar the material coughed up from the lungs, and especially, in recent cases, that which is coughed up upon first rising in the morning. This should be placed in the physician's hands as promptly as possible; although a delay of some days does not vitiate the result, and the tubercle bacilli may still be demonstrated after the sputum has undergone putrefaction. It is well to pour the specimen into a clean, shallow vessel having a blackened bottom—a Petri's dish placed upon a piece of dead-black paper will answer very well. In tuberculous sputum small, lenticular masses of a yellowish color may usually be observed, and one of these should be selected for microscopical examination, by picking it up with a platinum needle and freeing it as far as possible from the tenacious mucus in which it is embedded. If such masses are not recognized take any purulent-looking material present in the specimen, whether it be in small specks distributed through the mucus, or in larger masses. A little of the selected material should be placed in the centre of a clean cover glass and another thin glass cover placed over it. By pressure and a to-and-fro motion the material is crushed and distributed as evenly as possible; the glasses are then separated by a sliding motion. The film is permitted to dry by exposure in the air. When dry the cover glass, held in forceps, is passed three times through the flame of an alcohol lamp or Bunsen burner to fix the albuminous coating. Too much heat causes the film to turn brown and ruins the preparation. The staining fluid (Ziehl's carbol-fuchsin) may then be poured upon the cover glass, or this may be floated upon the surface of the fluid contained in a shallow watch glass. Heat is now applied by bringing the cover glass over a flame and holding it there until steam begins to be given off from the surface of the staining fluid; it is then withdrawn and again gently heated at intervals for a minute or two. The cover glass is then washed in water, and the film will be seen to have a uniform deep-red color. The next step consists in decolorization in the acid solution (twenty-five-per-cent solution of nitric or of sulphuric acid). The cover glass is gently moved about in this solution for a few seconds, and the color will be seen to quickly fade to a greenish tint. The object is to remove all color from the cells and the albuminous background, so that the bacilli, which retain their color in presence of the acid, may be clearly seen. The preparation is next washed in dilute alcohol (sixty per cent) to remove the fuchsin which has been set free by the acid. If decolorization was not carried far enough the film will be seen to still have a red color, especially in places where it is thickest, when it is removed from the dilute alcohol and washed out in water. In this case it will be necessary to return it to the acid solution and again wash it in the dilute alcohol and in water. It may now be placed in a solution of methylene blue or of vesuvin for a contrast stain. The tubercle bacilli are distinguished by the fact that they retain the red color imparted to them in the fuchsin solution, while other bacteria present, having been decolorized in the acid solution, take the contrast stain and appear blue or brown, according to the color used. The double-stained preparation, after a final washing in water, may be examined at once, or dried and mounted in balsam for permanent preservation.

Of the various other methods which have been proposed, that of Fränkel, as modified by Gabbett, appears to be the most useful. This consists in staining as above directed with Ziehl's carbol-fuchsin solution, and in then placing the cover glass directly in a second solution which contains both the acid for decolorizing and the contrast stain. This second solution contains twenty parts of nitric acid, thirty parts of alcohol, fifty parts of water, and sufficient methylene blue to make a saturated solution (one to two parts in one hundred). After remaining in this solution for a minute or two the cover glass is washed in water, and upon microscopical examination the tubercle bacilli, if present, will be seen as red rods which strongly contrast with the blue background.

The methods recommended for cover-glass preparations may also be used for staining the tubercle bacillus in thin sections of tuberculous tissues, except that it is best not to employ heat. The sections may be left for an hour in the carbol-fuchsin solution, or for twelve hours in the Ehrlich-Weigert tubercle stain—eleven cubic centimetres of saturated alcoholic solution of methyl violet, ten cubic centimetres of absolute alcohol, one hundred cubic centimetres of aniline water. They should then be decolorized by placing them for about half a minute in dilute nitric acid (ten per cent); then wash out color in sixty-per-cent alcohol; counter-stain for two or three minutes in a saturated aqueous solution of methylene blue; dehydrate with absolute alcohol or with aniline oil; clear up in oil of cedar, and mount in xylol balsam. If the aniline-water-methyl-violet solution has been used for staining the bacilli a saturated solution of vesuvin may be used as a contrast stain.

Biological Characters.—A *parasitic, aërobic, non-motile* bacillus, which grows only at a temperature of about 37° C. Is also a *facultative anaërobic* (Fränkel).

The question as to *spore formation* has not been definitely determined. It has been generally assumed that the unstained spaces which are frequently seen in the bacilli are spores; and the fact that caseous material in which a microscopical examination has failed to demonstrate the presence of bacilli may produce tuberculosis, with bacilli, when inoculated into guinea-pigs, has been explained upon the supposition that this material contained spores. But a few bacilli present in such caseous material might easily escape detection. As pointed out by Fränkel, the oval spaces in stained specimens have not the sharply defined outlines of spores. Moreover, the bacilli, when examined in unstained preparations, do not contain corresponding refractive bodies, recognizable as spores. And when the bacilli are stained by Gram's method the protoplasm is often contracted in the form of little, spherical stained masses, while the unstained spaces are larger and no longer have the oval form presented in rods stained by Ehrlich's method. The great resisting power of the bacillus to heat and to desiccation has been supposed to be due to the presence

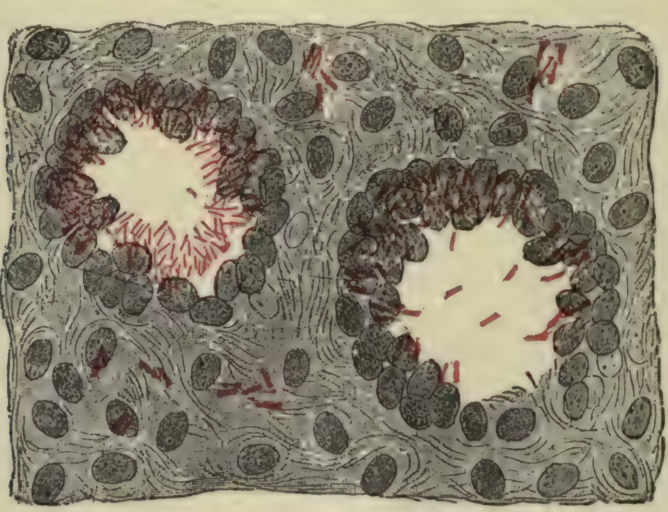

FIG. 116.—Section through a tuberculous nodule in the lung of a cow, showing two giant cells containing tubercle bacilli. × 950. (Baumgarten.)

of spores. But, so far as resistance to heat is concerned, this is not so great as was at one time believed. Schill and Fischer (1884), assuming that the tubercle bacillus forms spores, made quite a number of experiments to determine its thermal death-point. They subjected sputum containing the bacillus to a temperature of 100° C., and tested the destruction of vitality by inoculations into guinea-pigs. Exposure to steam at a temperature of 100° C. for two to five minutes was effective in every experiment, with one exception. One guinea-pig died tuberculous after having been inoculated with sputum exposed to this temperature for two minutes. This result was assumed to show that the bacillus would survive lower temperatures, but it is evident that additional experiments were required to establish this fact. In 1887 the writer made a few similar experiments at a lower temperature, and guinea-pigs inoculated with tuberculous sputum exposed for ten minutes to a temperature of 90°, 80°, and 60° C. failed to become tuberculous, while another guinea-pig, inoculated with the same material after exposure to a temperature of 50° C. for ten minutes, died tuberculous. These results correspond with those subsequently (1888) reported by Yersin, who tested the thermal death-point of this bacillus by the culture method. This author assumes that the bacilli form spores, but states as a result of his experiments that "at the end of ten days bacilli heated for ten minutes at 55° C. gave a culture in glycerin-bouillon ; those heated to 60°, at the end of twenty-two days; while those heated to 70° and above failed to grow in every instance. This experiment, repeated a great number of times, always gave the same result. The tubercle bacilli then resist a temperature of 60° C. for ten minutes, and it is to be remarked that the resistance of spores to heat appears to be no greater than that of the bacilli themselves." Yersin remarks in a footnote that "the spores which served for these experiments did not appear as more or less irregular granules taking the coloring matter strongly, but as veritable spores with sharply defined outlines, to the number of one or two in a bacillus, or three at the outside. These spores are particularly clear in cultures upon glycerin-agar several weeks old."

It may be that bacteriologists have been mistaken in the inference that all spores possess a greater resisting power for heat than that exhibited by bacilli in the absence of spores. That this is true as regards anthrax spores and many others, the thermal death-point of which has been determined by exact experiments, does not prove that it is true for all. And it is known that there are wide differences in the resisting power both of the spores of different species and in the vegetating cells. To admit that the tubercle bacillus or the typhoid bacillus, etc., may form spores which have no greater resisting power against heat than the bacilli themselves, would therefore simply be an admission that some bacteriologists had made a mistaken inference based upon incomplete data. In view of the facts stated we can simply repeat what was said at the outset, viz., the question as to spore formation has not been definitely determined.

The tubercle bacillus is a *strict parasite*, and its biological characters are such that it could scarcely find natural conditions, outside of the bodies of living animals, favorable for its multiplication. It therefore does not grow as a saprophyte under ordinary circumstances. But it has been noted by Roux and Nocard that when it has been cultivated for a time in artificial media containing glycerin it may grow in a plain bouillon of veal or chicken, in which media it fails to develop when introduced directly from a culture originating from the body of an infected animal. This would indicate the possibility of its acquiring the ability to grow as a saprophyte ; and we can scarcely doubt that at some time in the past it was a true saprophyte. The experiments of Nuttall indicate that the bacillus may multiply, under favorable temperature conditions, in tuberculous sputum outside of the body. And it is extremely probable that multiplication occurs in the muco-purulent secretion which accumulates in pulmonary cavities in phthisical patients. In these cavities its development may, in a certain sense, be regarded as saprophytic, as it feeds upon non-living organic material.

Koch first succeeded in cultivating this bacillus upon coagulated *blood serum*, prepared as directed in Section VIII., Part First, of the present volume. Roux and Nocard have since shown (1888) that it grows very well on nutrient agar to which glycerin has been added (six to eight per cent), and also in veal broth containing five per cent of glycerin. It is difficult to obtain pure cultures from tuberculous sputum, on account of the presence of other bacteria which grow much more rapidly and take full possession of the medium before the tubercle bacillus has had time to form visible colonies. For this reason it is best to first inoculate a guinea-pig with the tuberculous sputum and to obtain cultures from it after tuberculous infection has fully developed. The inoculated animals usually die at the end of three or four weeks. It is best to kill one which gives evidence of being tuberculous, and to remove one or more nodules from the lungs through an opening made in the chest walls. The greatest care will be required to prevent contamination by other common microörganisms. The instruments used must be sterilized by heat, and the skin over the anterior thoracic wall carefully turned back ; then, after again sterilizing knives and scissors, cut an opening into the chest cavity, draw out the root of the lung, and take up with slender sterilized forceps, or with a strong platinum loop, one or more well-defined tubercular nodules. These may be conveyed directly to the surface of the solid culture medium and then broken up and rubbed over the surface as thoroughly as possible; or they may first be crushed between two sterilized glass slides, and then transferred with the platinum loop and thoroughly rubbed into the surface of the culture medium.

This breaking-up of the tuberculous nodules and distribution of the bacilli upon the surface of the culture medium is essential for the success of the experiment. Instead of using the tubercular nodules in the lungs, an enlarged lymphatic gland from the axilla or elsewhere may be used, as first recommended by Koch. This is to be crushed in the same way; and it will be best to inoculate a number of tubes at the same time, as accidental contamination or failure to develop is very liable to occur in a certain number. Owing to the liability of the blood serum to become too dry for the development of the bacillus, it is best to keep the cultures in a moist atmosphere, or to prevent evaporation by applying a rubber cap over the open end of the test tube. This should be sterilized in a solution of mercuric chloride (1 : 1,000); and the end of the cotton plug should be burned off just before applying it, for the purpose of destroying the spores of mould fungi, which in a dry atmosphere would be harmless, but under the rubber cap are likely to sprout and to send their mycelium through the cotton plug to the interior of the tube, thus destroying the culture.

Upon coagulated blood serum the growth first becomes visible at the end of ten to fourteen days (at 37° C.), and at the end of three weeks a very distinct and characteristic development has occurred. The first appearance is that of dry-looking, grayish-white points and scales, which are without lustre, and are sometimes united to form a thin, irregular, membranous-looking layer. Under the microscope, with an amplification of eighty diameters, the early, thin surface growth upon blood serum presents a characteristic appearance. The bacilli, arranged in parallel rows, form variously curved figures, of which we may obtain impressions by carefully applying a dry cover glass to the surface. Upon staining the preparation in the usual way the same arrangement of the bacilli which adhered to the thin glass cover will be preserved. The growth is more abundant in subsequent cultures, which have been kept up in Koch's laboratory from his original pure cultures up to the present time; in these the bacillus still pre-

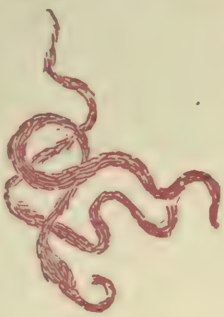

FIG. 117.—Tubercle bacilli from surface of culture upon blood serum. × 500. (Koch.)

serves its characters of form and growth, and its specific pathogenic power.

Pastor (1892) has succeeded in obtaining pure cultures of the tubercle bacillus from sputum by the following ingenious method: After proving by microscopic examination that the sputum of a tuberculous individual contains numerous bacilli, he has the patient cleanse his mouth as thoroughly as possible with sterilized water, and then expectorate some material, coughed up from the lungs, into a sterilized test tube. By shaking with sterilized water a fine emulsion is made, and this is filtered through fine gauze. The filtrate, which is nearly transparent, contains numerous tubercle bacilli. A few drops of the emulsion are now added to liquefied gelatin in a test tube, and a plate is made in the usual way. This is kept for three or four days at the room temperature, during which time the common mouth bacteria capable of growth form visible colonies. By means of a hand lens a place is now selected in which no colonies are seen, and a bit of gelatin is excised with a sterilized knife. This piece is transferred to the surface of blood serum or glycerin-agar, and placed in the incubating oven, where in due time colonies of the tubercle bacillus will usually be found to develop.

Another method of accomplishing the same result has been described by Kitasato. This is a method devised by Koch some time since and successfully employed in his laboratory. The morning expectoration of a tuberculous patient, raised from the lungs by coughing, is received in a Petri's dish. A bit of sputum, such as comes from the tuberculous cavity in the lungs of such a patient, is now isolated with sterilized instruments and carefully washed in at least ten successive portions of sterilized water. By this procedure the bacteria accidentally attached to the viscid mass of sputum during its passage through the mouth are washed away. In the last bath the mass is torn apart and a small portion from the interior is used to make a microscopic preparation, the examination of which shows whether only tubercle bacilli are present. If this be the case cultures upon glycerin-agar are started from material obtained from the interior of the same mass. The colonies obtained in this way appear in about two weeks as round, white, opaque, moist, and shining masses. Kitasato's researches show that the greater portion of the tubercle bacilli in sputum obtained in this way, and in the contents of lung cavities, are incapable of development, although this fact cannot be recognized by a microscopic examination of stained specimens.

On account of the greater facility of preparing and sterilizing *glycerin-agar*, and the more rapid and abundant development upon this medium, it is now usually employed in preference to blood serum. The growth at the end of fourteen days is more abundant than upon blood serum at the end of several weeks. When numerous bacilli have been distributed over the surface of the culture medium a rather uniform, thick, white layer, which subsequently acquires a yellowish tint, is developed; when the bacilli are few in number or are associated in scattered groups separate colonies are developed, which acquire considerable thickness and have more or less irregular outlines; they are white at first, then yellowish-white. Fränkel describes the tubercle bacillus as a facultative anaërobic, and it would appear that it must be able to grow in situations where it can obtain very little oxygen from its development in the interior of tuberculous nodules, lymphatic glands, etc. But in stick cultures in glycerin-agar development only occurs near the surface, and not at all in the deeper portion of the medium. In view of its abundant growth on the surface it is difficult to understand this failure to grow along the line of puncture, if it is in truth a facultative anaërobic.

In peptonized veal broth containing five per cent of glycerin the bacillus develops at first in the form of little flocculi, which accumulate at the bottom of the flask and which by agitation are easily broken up. At the end of two or three weeks the bottom of the flask is covered with similar flocculi, which form an abundant deposit.

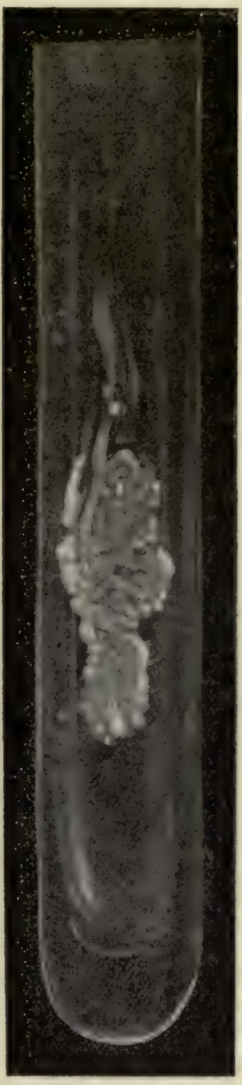

FIG. 118.—Culture of tubercle bacillus upon glycerin-agar. Photograph by Roux.

Pawlowski and others report success in cultivating the tubercle bacillus upon the surface of cooked *potato* enclosed in a test tube after the method of Bolton and Roux. The open end of the tube is hermetically sealed in a flame after the bacilli have been planted upon the obliquely-cut surface of the potato; this prevents drying. According to Pawlowski, better results are obtained if the surface of the potato is moistened with a five-per-cent solution of glycerin. The growth is said to be seen at the end of about twelve days as grayish, dry-looking flakes; at the end of three or four weeks it forms a dry, smooth, whitish layer, and no further development occurs.

The range of temperature at which this bacillus will grow is very restricted; 37° C. is usually given as the most favorable point, but Roux and Nocard say that the most favorable temperature appears to be 39°, and that development is slower at 37°.

The experiments of Koch, Schill and Fischer, and others show that the bacilli retain their vitality in desiccated sputum for several months (nine to ten months—De Toma); but they are said to undergo a gradual diminution in pathogenic virulence, which is more rapid when the desiccated material is kept at a temperature of 30° to 40° C. In the experiments of Cadéac and Malet portions of the lung from a tuberculous cow, dried and pulverized, produced tuberculosis in guinea-pigs at the end of one hundred and two days. They retain their vitality for a considerable time in putrefying material (forty-three days—Schill and Fischer ; one hundred and twenty days—Cadéac and Malet). The resisting power of this bacillus against germicidal agents is also greater than that of certain other pathogenic microörganisms, but not so great as to justify the inference that it forms spores. It is not destroyed by the gastric juice in the stomach, as is shown by successful infection experiments in susceptible animals, by mixing cultures of the bacillus with their food (Baumgarten, Fischer), and also by experiments with an artificially prepared gastric juice (Falk). They are destroyed, in sputum, in twenty hours by a three-per-cent solution of carbolic acid, even when they present the appearance usually ascribed to the presence of spores (Cavagnis) ; also by absolute alcohol, a saturated aqueous solution of salicylic acid, saturated aniline water, etc. (Schill and Fischer). The more recent experiments of Yersin upon pure cultures of the bacillus gave the following results : "Tubercle bacilli, containing spores, were killed by a five-per-cent solution of carbolic acid in thirty seconds, by one-per-cent in one minute ; absolute alcohol, five minutes ; iodoform-ether, one per cent, five minutes ; ether, ten minutes ; mercuric chloride, 1 : 1,000 solution, ten minutes ; thymol, three hours ; salicylic acid, 2.5 per cent, six hours.

The tubercle bacillus appears to be especially susceptible to the action of light. In his address before the Tenth International Medical Congress (Berlin, 1890) Koch says that when exposed to direct sunlight the tubercle bacillus is killed in from a few minutes to several hours, according to the thickness of the layer ; it is also destroyed by diffuse daylight in from five to seven days when placed near a window. This fact has an important hygienic bearing, especially in view of the fact that the tubercle bacillus is not readily killed by desiccation, putrefaction of the material containing it, etc. Tuberculous sputum expectorated upon sidewalks, etc., being exposed to the action of direct sunlight, will in many cases be disinfected by this agent by the time complete desiccation has occurred—*i.e.*, before it is in a condition to be carried into the air as dust.

Sawizky in 1891 made a series of experiments to determine the length of time during which dried tuberculous sputum retains its virulence. He arrived at the conclusion that virulence is not suddenly but gradually lost, and that in an ordinary dwelling room dried sputum retains its specific infectious power for two and one-half months.

Tizzoni and Cattani (1892) have presented some experimental evidence which indicates that injections of Koch's tuberculin into guinea-pigs may produce in these animals a certain degree of immunity against tuberculosis; and that this immunity depends upon the presence of an anti-tuberculin formed in the body of the partially immune animal.

Numerous experiments made by veterinary surgeons upon tuberculous cows show that the injection of Koch's tuberculin in these animals, in doses of thirty to forty centigrammes, produces a rise of temperature of from 1° to 3° C. The febrile reaction usually occurs in from twelve to fifteen hours after the injection. Its duration and intensity do not depend upon the extent of the tuberculous lesions, but is even more marked when these are slight than in advanced cases. In non-tuberculous animals no reaction occurs, and the experiments made justify the suspicion that tuberculosis exists if an elevation in temperature of a degree or more occurs as a result of the subcutaneous injection of the dose mentioned.

When the number of tubercle bacilli in sputum is comparatively small they may easily escape observation. Methods have therefore been suggested for finding them under these circumstances. Ribbert (1886) proposed the addition to the sputum of a two-per-cent solution of caustic potash, and boiling the mixture. The tenacious mucus is dissolved, and when the mixture is placed in a conical glass vessel the bacilli are deposited at the bottom and may easily be found in the sediment after removing the supernatant fluid. The same object is accomplished by Stroschein (1889) by the addition to sputum of three times its volume of a saturated solution of borax and boracic acid in water.

A method of *estimating the number* of bacilli in sputum has been proposed by Nuttall, which appears to give sufficiently accurate results and to be useful in judging of the progress of a case or of the results of treatment. For the details of this method we must refer to the author's paper (Johns Hopkins Hospital *Bulletin*, vol. xi., No. 13, 1891). It consists essentially in first making the sputum fluid by the addition of a solution of caustic potash; in then shaking it thoroughly in a bottle containing sterilized gravel or pounded glass; in carefully measuring the total quantity of fluid, and in dropping upon glass slides uniform drops by means of a graduated pipette; in spreading these uniformly by means of a platinum needle and a turn table; in covering the dried film with a film of blood serum, and coagulating this by heat; and, finally, in staining and counting the bacilli in a series of slides from the same specimen, and from the average number found in a single drop estimating the total number in the sputum for twenty-four hours.

Pathogenesis.—Man, cattle, and monkeys are most subject to contract the disease naturally, and it may be communicated by inoculation to many of the lower animals—guinea-pigs, field mice, rabbits, and cats are among the most susceptible animals; and in larger doses dogs, rats, white mice, and fowls may also be infected.

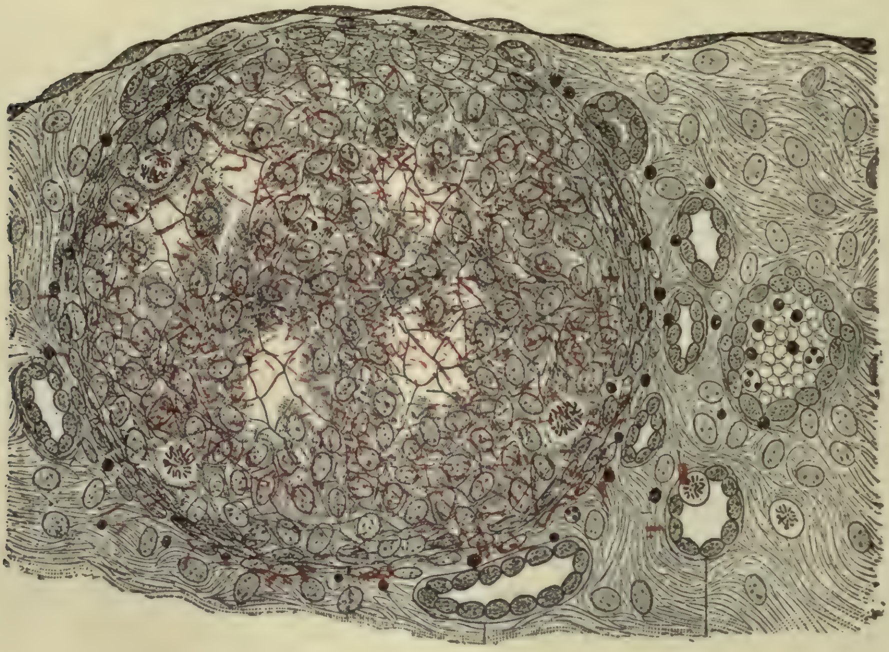

FIG. 119.—Limited epithelioid celled tubercle of the iris. × 950. (Baumgarten.)

When tuberculous sputum is introduced beneath the skin of a guinea-pig the nearest lymphatic glands are found to be swollen at the end of two or three weeks, at the same time there is a thickening of the tissues about the point of inoculation; later a dry crust forms over the local tuberculous tumefaction, and beneath this is a flattened ulcer covered with cheesy material. The animals become emaciated and show difficulty in breathing, and usually succumb to general tuberculosis, especially involving the lungs, within four to eight weeks. Injections of tuberculous sputum, or of pure cultures of the bacillus, into the peritoneal cavity give rise to extensive tuberculosis of the liver, spleen, and lungs, and to death, as a rule, within three or four weeks. Rabbits are less susceptible to subcutaneous injections, but die within seventeen to twenty days when virulent—recent—cultures are injected into the circulation. As a result of such an inoculation the animal rapidly loses flesh and has a decided elevation of temperature, commencing at the end of the first week and increasing considerably during the last days of life. At the autopsy the spleen and liver are found to be greatly enlarged, but they do not contain any tubercles that can be recognized by the naked eye (Yersin). They contain, however, great numbers of tubercle bacilli, both free and in the cells. Injections of a small quantity of a pure culture into the anterior chamber of the rabbit's eye cause first iris-tuberculosis, followed by swelling and caseation of the nearest lymph glands, and finally general infection and death ; when larger quantities are injected general tuberculosis is quickly developed. The influence of quantity—number of bacilli—is also shown in subcutaneous, intravenous, or intraperitoneal injections into guinea-pigs and rabbits (Hirschberger, Gebhardt, Wyssokowitsch). Thus rabbits which received less than one hundred and fifty bacilli, in sputum, in the experiments of Wyssokowitsch, did not develop tuberculosis ; and in guinea-pigs the smaller the number injected the more protracted the course of the disease was found to be.

Tuberculosis in man no doubt results, in a large proportion of the cases, from the respiration, by a susceptible individual, of air containing the tubercle bacillus in suspension in a desiccated condition. As already stated, it has been demonstrated by experiment that the bacillus retains its vitality in desiccated sputum for several months. The experiments of Cornet have demonstrated that in the dust of apartments occupied by tuberculous patients tubercle bacilli are very commonly present in sufficient numbers to induce tuberculosis in guinea-pigs inoculated in the peritoneal cavity with such dust, while negative results were obtained from inoculations with dust from other localities. In view of these facts the usual mode of infection is apparent. Infection may also occur through an open wound or abrasion of the skin, as in the small, circumscribed tumors which sometimes develop upon the hands of pathologists as a result of handling tuberculous tissues. A few instances of accidental inoculation through wounds made by glass or earthen vessels containing tuberculous sputum have also been recorded. A more common mode of infection, especially in children, is probably by way of the intestinal glands, from the ingestion of the milk of tuberculous cows. That infection may occur by way of the intestine has been proved by experiments upon rabbits, which develop tuberculosis when fed upon tuberculous sputum. And that the tubercle bacillus is frequently, if not usually, present in the milk of tuberculous cows has been proved by the experiments of Bollinger, Hirschberger, Ernst, and others.

In Hirschberger's investigations milk from tuberculous cows induced tuberculosis in guinea-pigs, when injected subcutaneously or into the peritoneal cavity, in fifty-five per cent of the cases studied (twenty). The conclusion is reached that the milk may contain tubercle bacilli even when the udder of the cow is not involved. Ernst also, from an examination of the milk from thirty-six tuberculous cows in which the udder was apparently not involved, found the tubercle bacillus by microscopical examination in five per cent of the samples examined (one hundred and fourteen).

The prevalence of tuberculosis among cattle is shown by numerous investigations, and especially by the official inspections of slaughtered animals made in Germany. Thus in Saxony, in the year 1889, of 611,511 cattle examined 6,135 were found to be tuberculous (about one per cent); in Berlin, 1887–1888, out of 130,733 animals slaughtered 4,300 were found to be tuberculous (3.2 per cent). In view of the facts stated the great mortality from tubercular diseases among children, many of whom are removed from other probable sources of infection, is not difficult to understand, and the practical and simple method of preventing infection in this way, afforded by the sterilization (by heat) of milk used as food for infants, must commend itself to all.

BACILLUS TUBERCULOSIS GALLINARUM.

The researches of Maffucci (1889) and of Cadiot, Gilbert, and Roger (1890) show that the bacillus obtained from spontaneous tuberculosis in chickens, although closely resembling the bacillus of human tuberculosis, is not identical with it, varying especially in its pathogenic power. This view is sustained by the observations of Koch, who says in his address before the Tenth International Medical Congress (Berlin, 1890):

> "The care which it is necessary to exercise in judging of the characters which serve to differentiate bacteria, even those which are well known, I have learned in the case of the tubercle bacillus. This species is so definitely characterized by its staining reactions, its growth in pure cultures, and its pathogenic qualities, and indeed by each of these characters, that it seems impossible to confound it with other species. Nevertheless in this case also one should not rely upon a single one of the characters mentioned for determining the species, but should follow the safe rule that all available characters should be considered, and the identity of a certain bacterium should only be regarded as demonstrated when it has been shown to correspond in all of these particulars. When I made my first researches with reference to the tubercle bacillus I was controlled by this rule, and tested tubercle bacilli from various sources, not only with reference to their staining reactions, but also with reference to their growth in culture media and pathogenic characters. Only in the tuberculosis of chickens I was not able to apply this rule, as at that time it was not possible for me to obtain fresh material from which to make pure cultures. As, however, all other forms

of tuberculosis had given identical bacilli, and the bacilli of chicken tuberculosis in their appearance and behavior towards the aniline colors entirely corresponded with these, I believed myself justified in assuming their identity, notwithstanding the incompleteness of the research. Later I received pure cultures from various sources, which apparently originated from tubercle bacilli, but in several regards differed from these; especially in the fact that inoculation experiments, made by experienced and reliable investigators, led to dissimilar results, which it was necessary to regard as unexplained contradictions. At first I believed that these differences depended upon changes such as are frequently observed in pathogenic bacteria, when these are cultivated in pure cultures outside of the body for a long time under more or less unfavorable conditions. In order to solve the riddle I attempted by various influences to change the common tubercle bacilli into the presumed variety referred to. They were cultivated for several months at so high a temperature that only a scanty growth was obtained; in other experiments still higher temperatures were allowed to act repeatedly for so long a time that the cultures were brought as nearly as possible to the point of killing the bacilli. In a similar way I subjected the cultures to the action of chemical agents, of light, or absence of moisture; they were cultivated for many generations in association with other bacteria; inoculated successively in animals having but a slight susceptibility. But, in spite of all these attempts, only slight variations were obtained in their characters—far less than other pathogenic bacteria undergo under similar circumstances. It appears, therefore, that the tubercle bacilli retain their characters with special obstinacy; this is in accord with the fact that pure cultures which have now been cultivated by me in test tubes for more than nine years, without in the meantime having been in a living body, are still entirely unchanged with the exception of a slight diminution of virulence. . . . It happened about a year ago that I received a living chicken which was suffering from tuberculosis, and I used this opportunity to make cultures directly from the diseased organs of this animal, which previously I had not been able to do. When the cultures grew I saw to my surprise that they had precisely the appearance and all of the characters possessed by the enigmatical cultures resembling those of the genuine tubercle bacillus. Later I learned that these also originated from tuberculosis in fowls, but, upon the assumption that all forms of tuberculosis are identical, had been considered genuine tubercle bacilli. A verification of my observations I find in the recently published researches of Prof. Maffucci with reference to tuberculosis of fowls."

According to Maffucci, adult chickens are refractory against the action of the Bacillus tuberculosis from man, and there are slight morphological and biological differences in the bacilli from the two sources.

Cadiot, Gilbert, and Roger (1891) have made a series of experiments with the bacillus of tuberculosis in fowls. They found the bacilli to be very numerous in the livers of chickens suffering from spontaneous tuberculosis, and inoculated with material from this source six chickens, five rabbits, and twelve guinea-pigs. The chickens, when inoculated in the cavity of the abdomen or by injection into a vein, died in from forty-one to ninety-three days from general tuberculosis. Four of the rabbits died of general tuberculosis, presenting the same appearance as that following inoculation with bacilli from human tuberculosis. Of the guinea-pigs, which were inoculated in the cavity of the abdomen, eleven remained in good health and one only died of general tuberculosis. These experiments show a decided difference in the pathogenic properties of tubercle bacilli from the two sources, for the guinea-pig is especially susceptible to tuberculosis as a result of similar inoculations with bacilli from human tuberculosis. We must therefore conclude that the bacillus found in spontaneous tuberculosis in fowls is a distinct variety of Bacillus tuberculosis. Whether this variety would cause tuberculosis in man, if introduced into susceptible subjects, has not been determined; and, as pointed out by Koch, this question can only be answered in the affirmative if it should be obtained in pure cultures from cases of human tuberculosis.

Since the above was written Maffucci has published (1892) an elaborate memoir upon tuberculosis of fowls. His conclusions are stated as follows:

> "The bacillus of tuberculosis in fowls is distinguished from that of tuberculosis in mammals by the following points of difference:
>
> "1. It does not induce tuberculosis in guinea-pigs, and seldom causes general tuberculosis in rabbits.
>
> "2. The cultures in various media have a different appearance from those of the Bacillus tuberculosis of mammals.
>
> "3. The temperature at which it develops varies between 35° and 45° C., and the thermal death-point is 70° C.
>
> "4. At 45° to 50° C. the cultures show long, thick, and branched forms.
>
> "5. The bacillus retains its vegetative and pathogenic power at the end of two years.
>
> "6. This bacillus produces a substance which is toxic for guinea-pigs and is but slightly toxic for grown fowls.
>
> "7. The tuberculosis produced in fowls by this bacillus is without giant cells."

Additional Notes upon the Tubercle Bacillus (1895).—Several authors (Metschnikoff, Czaplewski, Fischel) have described branching forms of the tubercle bacillus, and Lubinsky (1895) reports that in certain media it grows out into long threads, which, however, he has never observed to be branched. The media used by him are said to give a more abundant growth than occurs upon glycerin-agar; the most favorable being made of flesh-peptone agar, or flesh-peptone bouillon, containing four per cent of glycerin and mashed potato, one kilo of finely chopped and washed potato to fifteen hundred cubic centimetres of water; this is cooked for three or four hours and filtered —to the filtrate is added four per cent of glycerin; one and a half per cent of agar is now added and the mixture is again cooked and filtered.

Jones (1895) has observed the branching forms previously described by several authors, and states that they are only found upon the surface of culture media where there is free access of oxygen. He concludes that the tubercle bacillus does not form endogenous spores, such as are found in various other bacilli, but that in the rods and branched filaments certain objects are seen which are probably reproductive elements, and which closely resemble similar bodies ("Kolben") seen in the actinomyces fungus, to which Jones believes the tubercle bacillus is closely related.

Prudden and Hodenpyl (1891) have shown that the injection of dead tubercle bacilli in rabbits gives rise to the development of nodules in the lung containing epithelioid and giant cells, but that these never undergo caseation. This fact is supposed to justify the inference that caseation is due to the products elaborated during the growth of living tubercle bacilli. The results reported by Vissmann (1892) correspond with those reported by Prudden and Hodenpyl. Gamaléia (1892) has also obtained nodules with epithelioid and giant cells from the injection of dead tubercle bacilli, but in his experiments he also found caseation of the nodules. Baumgarten suggests that this was probably due to the fact that there were some living tubercle bacilli remaining in the cultures which he injected.

Loomis (1890) and Pizzini (1892) have shown that living tubercle bacilli are not infrequently found in the bronchial glands of individuals who present no evidence of tubercular disease of the lungs or elsewhere. The author last mentioned inoculated thirty guinea-pigs with the bronchial, mesenteric, and cervical glands of thirty individuals in whom death was due to accident or acute disease, and who were free from tuberculosis. Twelve of these thirty guinea-pigs developed tuberculosis as a result of the inoculation.

Straus (1894) has found tubercle bacilli in the nasal cavities of healthy individuals.

Ernst (1895), as the result of extended researches made under the auspices of the Massachusetts Society for Promoting Agriculture, has arrived at the following conclusions with reference to the presence of the tubercle bacillus in the milk of tuberculous cows:

"The possibility of milk from tuberculous udders containing the infectious element is undeniable.

"With the evidence here presented, it is equally undeniable that milk from diseased cows with no appreciable lesion of the udder may, and not infrequently does, contain the bacillus of the disease."

De Schweinitz (1894) has found that by continued cultivation in an artificial medium the tubercle bacillus becomes attenuated, so that when inoculated into guinea-pigs these animals give no evidence of tubercular infection for six months or more. And his experiments indicate that animals which have survived an inoculation with the attenuated tubercle bacillus acquire an immunity against the pathogenic action of virulent cultures.

Amann (1895) has given in the *Centralblatt für Bakteriologie* (Bd. xvii., page 513) a detailed account of his method for demonstrating the presence of tubercle bacilli in sputum by sedimentation. He mixes the sputum with two to four volumes of cold distilled water, in a glass cylinder which should not be more than half full. He adds one cubic centimetre of chloroform and a small quantity of shot; the glass cylinder is then closed with a rubber cork and violently shaken for some minutes. From four to six volumes of distilled water are then added and the mixture is placed in a V-formed glass tube for sedimentation; two cubic centimetres of carbol-fuchsin solution are added and distributed by gentle agitation of the tube. At the end of two days the sedimentation is complete and the stained bacilli, cells, connective-tissue fibres, etc., are taken up with a pipette for examination under the microscope.

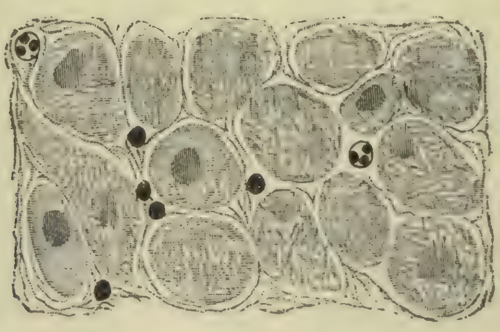

FIG. 120.—Section of a recent lepra nodule of the skin. × 950. (Baumgarten.)

BACILLUS LEPRÆ.

Discovered by Hansen (1879), chiefly in the interior of the peculiar round or oval cells found in leprous tubercles. Discovery confirmed by Neisser (1879) and by many subsequent observers.

While found chiefly in the leprous tubercles of the skin and mucous membranes, the bacilli have also been found in the lymphatic glands, the liver, the spleen, the testicles, and, in the anæsthetic form of the disease, in the thickened portions of nerves involved in the leprous process. Some observers have also reported finding them in the blood, but this appears to be quite exceptional. In the leprous cells they are commonly found in great numbers, and they may also be seen in the lymph spaces outside of these cells. They are not found in the epidermal layer of the skin, but, according to Babes, they may penetrate the hair follicles.

Morphology.—The bacillus of leprosy resembles the tubercle bacillus in form, but is of more uniform length and not so frequently bent or curved. The rods have pointed ends; and in stained preparations unstained spaces, similar to those observed in the tubercle bacillus and generally assumed to be spores, are to be seen, although not quite so distinctly as in the latter. The bacilli are said by Flügge to be from four to six μ in length and less than one μ in width—probably considerably less, for the same author states that the tubercle bacillus has about the diameter of the bacillus of mouse septicæmia, and this is given as $0.2\ \mu$.

This bacillus *stains* readily with the aniline colors and also by Gram's method. Although it differs from the tubercle bacillus in the ease with which it takes up the ordinary aniline colors, it resembles it in retaining its color when subsequently treated with strong solutions of the mineral acids. Double-stained preparations are therefore easily made by first staining sections or cover-glass preparations in Ziehl's carbol-fuchsin solution or in an aqueous solution of methyl violet, decolorizing in acid, washing in alcohol, and counter-staining with methylene blue—or, if methyl violet was used in the first instance, with vesuvin.

Biological Characters.—The earlier attempts to cultivate this bacillus were without success, but recently Bordoni-Uffreduzzi has obtained from the marrow of the bones of a leper a bacillus which he believes to be the leprosy bacillus, and which he was able to cultivate upon blood serum to which a certain amount of peptone and of glycerin had been added. At first this bacillus only grew with difficulty and in the incubating oven; but after it had been cultivated artificially through a number of generations it is said to have grown upon ordinary nutrient gelatin at the room temperature. The bacillus obtained in this way is said to have retained its color when treated with acids, after having been stained with aniline-fuchsin, corresponding in this respect with the bacillus of leprosy and the tubercle bacillus. But it differed considerably in its morphology from the Bacillus lepræ as seen in the tissues of lepers, being considerably thicker, and it was not so promptly stained by the aniline colors as is the bacillus found in the tissues. Moreover, attempts to cultivate the same bacillus from leprous tubercles of the skin were unsuccessful, as were also inoculation experiments into the anterior chamber of the eye in rabbits. It is therefore a matter of doubt as to whether the bacillus obtained by Bordoni-Uffreduzzi is identical with that present in such numbers in the cells of the leprous tubercles, to which the name Bacillus lepræ has been given.

Some of the earlier observers described the bacillus of leprosy as motile, but this assertion seems to have been based upon some error of observation, and it is now generally agreed that, like the tubercle bacillus, it is without proper movements. The question of spore formation has not been definitely settled. As before remarked, unstained portions, occurring at regular intervals, are seen in the rods in stained preparations ; but no satisfactory evidence has been presented to show that these are truly reproductive spores.

Pathogenesis.—The inference that the bacillus above described bears an etiological relation to the disease with which it is associated is based upon the demonstration of its constant presence in leprous tissues—which has now been repeatedly made in various and distant parts of the world—and of its absence from the same tissues involved in different morbid processes. As it has not been obtained in pure cultures, the final proof of such etiological relation is still wanting. We have, however, experimental evidence to show that leprous tissues containing this bacillus are infectious and may reproduce the disease. The experiment has been made upon man by Arning, who inoculated a condemned criminal subcutaneously with fresh leprous tubercles. The experiment was made in the Sandwich Islands, and the man was under observation until his death occurred from leprosy at the end of about five years. The first manifestations of the disease became visible in the vicinity of the point of inoculation several months after the experimental introduction of the infectious material.

Positive results have also been reported in the lower animals by Damsch, by Vossius, and by Melcher and Ortmann. The last-named investigators inoculated rabbits in the anterior chamber of the eye with portions of leprous tubercles excised for the purpose from a leper. The animals died from general infection at the end of several months, and the characteristic tubercles containing the bacillus were distributed through the various organs.

Wolters (1893) who has made numerous inoculation experiments and has made a critical review of all the recorded experimental evidence, arrives at the conclusion that the comparatively small number of successful results reported cannot be accepted as evidence that leprosy can be transmitted to the lower animals by inoculation. He believes that in some cases the tubercle bacillus has been present in the material inoculated and that the infectious process following the inoculation was tuberculous and not leprous. In inoculations into the anterior chamber, in the eyes of rabbits, the considerable number of bacilli introduced with the leprous tissue remain and retain their staining properties, so that the bacilli originally introduced are found in the leucocytes of the inflammatory exudate or granulation tissue formed as a result of the introduction of foreign material. Wolters also doubts whether the few successful results reported in the cultivation of the lepra bacillus are trustworthy. He has never succeeded in his efforts to cultivate the bacillus.

BACILLUS MALLEI.

Synonyms.—The bacillus of glanders; Der Rotzbacillus, *Ger.;* Bacille de la morve, *Fr.*

Discovered by Löffler and Schütz (1882), and proved to be the cause of glanders by the successful inoculation of pure cultures. Found especially in the recent nodules in animals infected with glanders ; also in the same after ulceration, and in the discharge from the nostrils, pus from the specific ulcers, etc.; sometimes in the blood of infected animals (Weichselbaum).

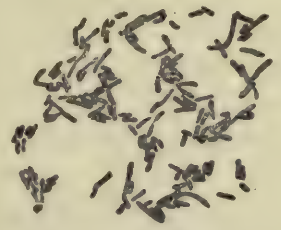

FIG. 121.—Bacillus mallei. × 1,000. From a photomicrograph. (Fränkel and Pfeiffer.)

Morphology.—Bacilli with rounded ends, straight or slightly curved, rather shorter and decidedly thicker than the tubercle bacillus ; usually solitary, but occasionally united in pairs, or in filaments containing several elements (in potato cultures). In stained preparations unstained or feebly stained spaces are seen in the rods, alternating with the deeply stained protoplasm of the cell. As in the tubercle bacillus, which presents a similar appearance, these spaces have been supposed by some bacteriologists to represent spores ; but Löffler believes them to represent rather a degeneration of the protoplasm. Baumgarten and Rosenthal claim to have demonstrated the presence of spores by the use of Neisser's method of staining, but they do not consider it established that the unstained spaces in the rods referred to are of this nature.

The glanders bacillus may be *stained* with aqueous solutions of the aniline colors, but the staining is more intense when the solution is made feebly alkaline. Add to three cubic centimetres of a 1:10,000 solution of caustic potash, in a watch glass, one cubic centimetre of a saturated alcoholic solution of an aniline color (methylene blue,

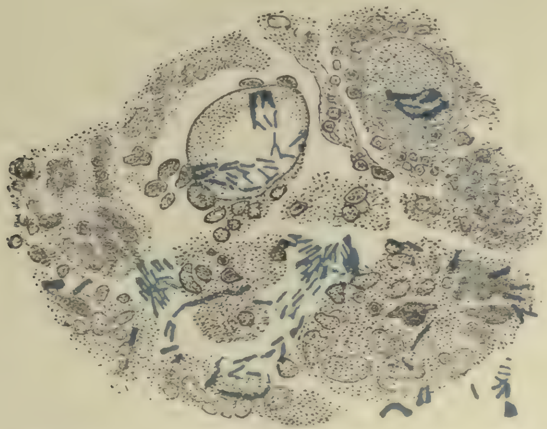

FIG. 122.—Section of a glanders nodule. × 700. (Flügge.)

gentian violet or fuchsin); or the aniline-water-fuchsin, or methyl violet solution of Ehrlich may be used, with the addition just before use of an equal quantity of 1 : 10,000 solution of caustic potash. Löffler recommends that cover-glass preparations be placed in Ehrlich's solution and heated for five minutes; then decolorized in a one-per-cent solution of acetic acid to which sufficient tropæolin has been added to give it the yellow color of Rhine wine ; then quickly washed in distilled water. This bacillus presents the peculiarity of losing very quickly in decolorizing solutions the color imparted to it by the aniline staining solutions. For this reason the staining of the bacillus in sections is attended with some difficulty. Löffler recommends his alkaline methylene-blue solution for staining sections ; and for decolorizing, a mixture containing ten cubic centimetres of distilled water, two drops of strong sulphuric acid, and one drop of a five-per-cent solution of oxalic acid. Thin sections should be left in this acid solution about five seconds. The method more recently recommended by Kühne also gives good results in skilful hands (see p. 35).

Biological Characters.—An *aërobic, non-motile, parasitic* bacillus, which may be cultivated in various artificial media at a temperature of 37° C. The lowest temperature at which development occurs (22° C.—Löffler) is a little above that at which nutrient gelatin is liquefied ; the highest limit is 43° C. According to Fränkel, the glanders bacillus is a *facultative anaërobic.* Baumgarten and Rosenthal claim to have demonstrated the presence of spores by Neisser's method of staining. Löffler was led to doubt the formation of spores from the results of his experiments upon the thermal death-point of this bacillus, and its comparatively slight resistance to desiccation and destructive chemical agents. He found that exposure for ten minutes to a temperature of 55° C., or for five minutes to a three- to five-per-cent solution of carbolic acid, or for two minutes to a 1 : 5,000 solution of mercuric chloride, was effectual in destroying its vitality. As a rule, the bacilli do not grow after having been preserved in a desiccated condition for a few weeks ; and in a moist condition the cultures cannot be preserved longer than three or four months—usually not so long as this (Löffler). The bacillus does not grow in infusions of hay, straw, or horse manure, and it is doubtful whether it finds conditions in nature favorable for its saprophytic existence. It grows, in the incubating oven, in neutral bouillon, in nutrient gelatin, or in nutrient agar, and still better in *glycerin-agar.* Upon the last-mentioned medium it grows, even at the room temperature (Kranzfeld), but better still in the incubating oven, as a pale-white, transparent streak along the line of inoculation, which at the end of six or seven days may have a width of seven to eight millimetres. According to Raskina, nutrient agar made with milk forms an extremely favorable medium, upon which a thick, pale-white layer develops in two or three days, which on the third or fourth day acquires an amber-yellow color, and the deeper layers acquire a brownish-red tint.

The growth upon solidified *blood serum*, in the course of three or four days at 37° C., consists of yellowish, transparent drops, which later coalesce into a viscid layer, which has a milky appearance from the presence of numerous small crystals (Baumgarten). The growth upon cooked *potato* is especially characteristic. In the incubating oven, at the end of two or three days, a rather thin, yellowish, transparent layer develops, which resembles a thin layer of honey. Later this ceases to be transparent, and the amber color changes, at the end of six to eight days, to a reddish-brown color; and outside of the reddish-brown layer, with more or less irregular outlines, the potato for a short distance acquires a greenish-yellow tint.

Pathogenesis.—Glanders occurs principally among horses and asses, but may be contracted by man from contact with infected animals; it has also been communicated, in one instance with a fatal result, by subcutaneous inoculation, resulting accidentally from the use of an imperfectly sterilized hypodermic syringe which had previously been used for injecting cultures of the bacillus into guinea-pigs. The field mouse and the guinea-pig are especially susceptible to infection by experimental inoculations; the cat and the goat may be infected in the same way. Lions and tigers in menageries are said to have contracted glanders from being fed upon the flesh of infected animals (Baumgarten). Rabbits have but slight susceptibility, and the same is true of sheep and dogs; swine, cattle, white mice, and common house mice are immune.

The etiological relation of the bacillus is fully established by the experiments of Löffler and Schütz, confirmed by other bacteriologists, which show that pure cultures injected into horses, asses, and other susceptible animals, produce genuine glanders. The disease is characterized in the equine genus by the formation of ulcers upon the nasal mucous membrane, which have irregular, thickened margins and secrete a thin, virulent mucus; the submaxillary lymphatic glands become enlarged and form a tumor which is often lobulated; other lymphatic glands become inflamed, and some of them suppurate and open externally, leaving deep, open ulcers; the lungs are also involved and the breathing becomes hurried and irregular. In farcy, which is a more chronic form of the same disease, circumscribed swellings, varying in size from a pea to a hazelnut, appear on different parts of the body, especially where the skin is thinnest; these suppurate and leave angry-looking ulcers with ragged edges, from which there is an abundant purulent discharge. The specific bacillus can easily be obtained in pure cultures from the interior of suppurating nodules and glands which have not yet opened to the surface, and the same material will give successful results when inoculated into susceptible animals. But the discharge from the nostrils or from an open ulcer contains comparatively few bacilli; and as these are associated with various other bacteria which grow more readily in our culture media, it is not easy to obtain pure cultures, by the plate method, from such material.

In the guinea-pig subcutaneous inoculation is followed in four or five days by tumefaction at the point of inoculation, and after a time a prominent tumor with caseous contents is developed; ulceration of the skin follows, and a chronic, purulent ulcer with irregular, indurated margins results; after a time this may cicatrize. Meanwhile the lymphatic glands become involved, and the symptoms of general infection are developed at the end of four or five weeks; the glands suppurate, and in males the testicles are also involved; finally a diffuse inflammation of the joints occurs, and death results from exhaustion. In the guinea-pig the specific ulcers upon the nasal mu-

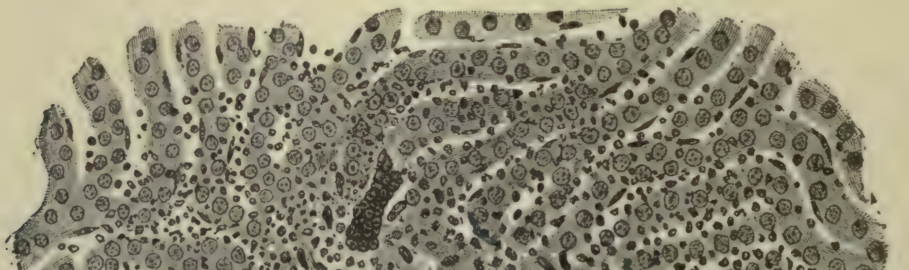

FIG. 123.—Section through a glanders nodule in liver of field mouse. Tissue × 250. Bacilli × 500. (Baumgarten.)

cous membrane, which characterize the disease in the horse, are rarely developed to any great extent.

In field mice general infection occurs at once as a result of the subcutaneous injection of a small quantity of a pure culture, and the animal dies at the end of three or four days. Upon post-mortem examination the principal changes are found in the liver and in the greatly enlarged spleen. Scattered through these organs are minute gray points which are scarcely visible to the naked eye. In the guinea-pig, which succumbs at a later date, these nodules are larger and closely resemble miliary tubercles, both macroscopically and under the microscope, in stained sections of the tissues. Similar nodules are also found in the kidneys and in the lungs; they have a decided tendency to undergo purulent degeneration. The bacilli are found principally in these nodules, of recent formation, and are commonly associated in groups, as if they had been enclosed in the interior of a cell the membranous envelope of which had undergone degeneration and disappeared.

As before remarked, it is not an easy matter to demonstrate the bacillus in sections of the tissues containing these nodules, owing to the facility with which they lose their color in alcohol and other decolorizing agents. For this reason it will be best to dehydrate sections by the use of aniline oil (Weigert's method) or to resort to Kühne's method of staining.

It is also difficult to demonstrate the presence of the bacillus in nodules which have undergone purulent degeneration, in the secretions from the nostrils of horses suffering from glanders, or in the pus from the specific ulcers and suppurating glands; for they are present in comparatively small numbers. But the virulent nature of these discharges is shown by inoculations into guinea-pigs or mice, and it is easier to obtain a pure culture from such virulent material by first inoculating a susceptible animal than directly by the plate method; for the small number of bacilli present, and their association with other bacteria which develop more rapidly in our culture media, make this a very uncertain procedure. For establishing the diagnosis of glanders, therefore, Löffler recommends the inoculation of guinea-pigs with pus from a suppurating gland or ulcer, or the nasal discharge from a suspected animal, rather than a direct attempt to demonstrate the presence of the bacillus by staining and culture methods.

The method proposed by Strauss gives more prompt results. This consists in the intraperitoneal injection of cultures or of the suspected products into the cavity of the abdomen of male guinea-pigs. If the glanders bacillus is present the diagnosis may be made within three or four days from the infectious process established in the testicles. At the end of this time the scrotum is red and shining, the epidermis desquamates, and suppuration occurs, the pus sometimes perforating the integument. This pus is found to contain the glanders bacillus. The animal usually dies in the course of twelve to fifteen days. When the animals are killed three or four days after the inoculation, the two layers of the tunica vaginalis testis are found to be covered with a purulent exudate containing the glanders bacillus and to be more or less adherent. Even as early as the second day the tunica vaginalis is seen to be covered with granulations.

An attenuation of virulence occurs in cultures which have been kept for some time, and inoculations with such cultures may give a negative result; or, when considerable quantities are injected, may produce a fatal result at a later date than is usual when small amounts of a recent culture are injected into susceptible animals.

Kalning, Preusse, and Pearson have obtained from cultures of the glanders bacillus a glycerin extract similar to the crude tuberculin of Koch—*mallein*. This, when injected into animals suffering from glanders, gives rise to a considerable elevation of temperature, and it is used as a means of diagnosis in cases of suspected infection in animals in which the usual symptoms have not yet manifested themselves. The value of the test has been demonstrated by numerous experiments.

Bonome (1894), as a result of extended researches, arrives at the following conclusions:

"1. The bacillus is found not only in the diseased tissues and purulent discharges, but also in the urine and milk of infected animals.

"2. The bacillus is found in the fœtus of infected animals even when the placenta is free from any pathological change.

"3. The glanders bacillus is very sensitive to desiccation and will not grow after being preserved for ten days at 25° C.

"4. In distilled water the bacillus dies out in six days.

"5. On the contrary, when protected from desiccation it resists a comparatively high temperature—70° C. for six hours; a temperature of 90° to 100° C. destroys it in three minutes."

BACILLUS OF LUSTGARTEN.

Synonym.—Syphilis bacillus.

Found by Lustgarten (1884) in syphilitic lesions and secretions of syphilitic ulcers, and believed by him to be the specific infectious agent in this disease. No satisfactory experimental evidence that this is the case has yet been obtained.

Morphology.—Straight or curved bacilli, which bear considerable resemblance to tubercle bacilli, but differ from them in the staining reactions. They are usually more or less curved, or bent at a sharp angle, or S-shaped;

the ends often present slight knob-like swellings; the length is from three and one-half μ to four and one-half μ, and the diameter is from 0.25 to 0.3 μ. With a high power the contour is seen to be not quite regular, but wavy in outline, and bright shining spaces in the deeply stained rods may be observed; these, from two to four in a single rod, are believed by Lustgarten to be spores. The bacilli are not found free in the tissues, but are enclosed in cells of a round-oval or polygonal form, which are said to be about double the size of a white blood corpuscle. The bacilli are not numerous, and very commonly only one or two are found in a single cell, but groups of six or eight may sometimes be seen, especially upon the margins of a syphilitic lesion, and in the tissues in the immediate vicinity of the infiltration, which show but little change or are apparently healthy (Lustgarten).

The presence of these bacilli in syphilitic lesions was demonstrated by Lustgarten by the following *staining method:* The thin sections are placed in the Ehrlich-Weigert gentian-violet solution (one hundred parts aniline water, eleven parts saturated alcoholic solution of gentian violet) for from twelve to twenty-four hours at the room temperature, and two hours in the incubating oven at 40° C. The sections are then thoroughly washed in alcohol and placed for ten seconds in a 1.5-per-cent solution of potassium permanganate; in this solution a precipitate of peroxide of manganese is formed, which adheres to the section; this is dissolved and washed off in a dilute aqueous solution of pure sulphuric acid; the sections are then washed in water, and, if not completely decolorized, are returned for a few seconds to the permanganate solution and again washed off in the acid; it may be necessary to repeat this operation three or four times. Finally the sections are dehydrated and mounted in balsam in the usual manner. Cover-glass preparations are made in the same way, except that, after being taken from the staining solution, they are washed off in water instead of in alcohol.

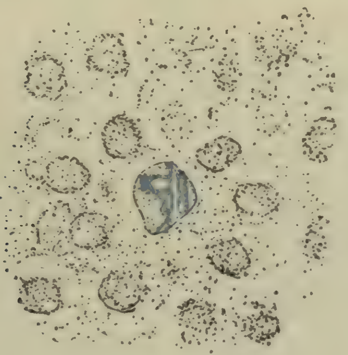

FIG. 124.

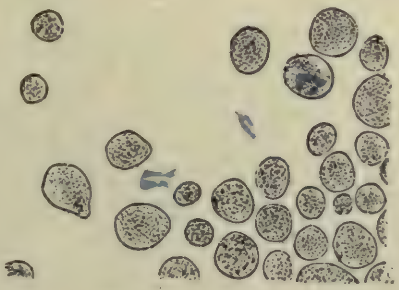

FIG. 125.

FIG. 124.—Migrating cell containing syphilis bacilli. (Lustgarten.)
FIG. 125.—Pus from hard chancre containing syphilis bacilli (Lustgarten.)

Another method of staining, recommended by De Giacoma, consists in placing the sections for twenty-four hours in aniline-water-fuchsin solution (cover-glass preparations may be stained in the same solution, hot, in a few minutes), then washing them in water, and decolorizing in a solution of perchloride of iron—first in a dilute and then in a saturated solution.

The method of staining employed by Lustgarten serves to differentiate his bacillus from many other microörganisms, but not from the tubercle bacillus and the bacillus of leprosy, which, as he pointed out, may be stained in the same way. And it has since been shown by Alvarez and Tavel, and by Matterstock, that in smegma from the prepuce or the vulva, bacilli are found which have the same staining reaction and are similar in their morphology to the bacillus of Lustgarten. This by no means proves that the smegma bacilli found under the prepuce of healthy persons are identical with the bacilli found by Lustgarten and others in sections of tissues involved in syphilomata. In the absence of pure cultures and inoculation experiments it is impossible to establish identity, however similar may be the characters referred to. Several well-known pathogenic bacilli resemble quite as closely in these particulars other bacilli which have, nevertheless, been differentiated from them by culture and inoculation experiments. We may mention especially in this connection the bacillus of diphtheria, as obtained from the pseudo-membranous exudation in a genuine case of this disease, and the pseudo-diphtheria bacilli found by Roux and Yersin in the fauces of healthy children. On the other hand, since it has been shown that similar bacilli are common in preputial smegma, we cannot attach great importance to the finding of Lustgarten's bacillus in primary syphilitic sores; and it has not been found in sufficient numbers, or with sufficient constancy, by those who have searched for it subsequently to the publication of Lustgarten's investigations, to give strong support to the view that it is the specific infectious agent in syphilis. Baumgarten, who has searched in vain for Lustgarten's bacillus in uncomplicated visceral syphilomata, suggests that the bacilli found occasionally in such lesions were perhaps tubercle bacilli and represented a mixed infection. As the bacillus under consideration has not been obtained in cultures, we have no information as to its biological characters and pathogenesis.

BACILLUS OF RHINOSCLEROMA.

First observed by Von Frisch (1882) in the newly formed tubercles of rhinoscleroma. Cultivated by Paltauf and Von Eiselberg (1886).

Rhinoscleroma is a chronic affection of the skin, and especially of the mucous membrane of the nares, which is characterized by the formation of tubercular thickenings of the skin and tumefaction of the nasal mucous membrane, followed sometimes by ulceration. It prevails in Italy, Austria, and to a slight extent in some parts of Germany. Pathologists generally regard it as an infectious process, although this has not been proved.

The bacilli, first described by Von Frisch, appear to be constantly present in the newly formed tubercles. They are commonly found in certain large hyaline cells peculiar to the disease, and may also be observed in the lymphatic vessels or scattered about in the involved tissues.

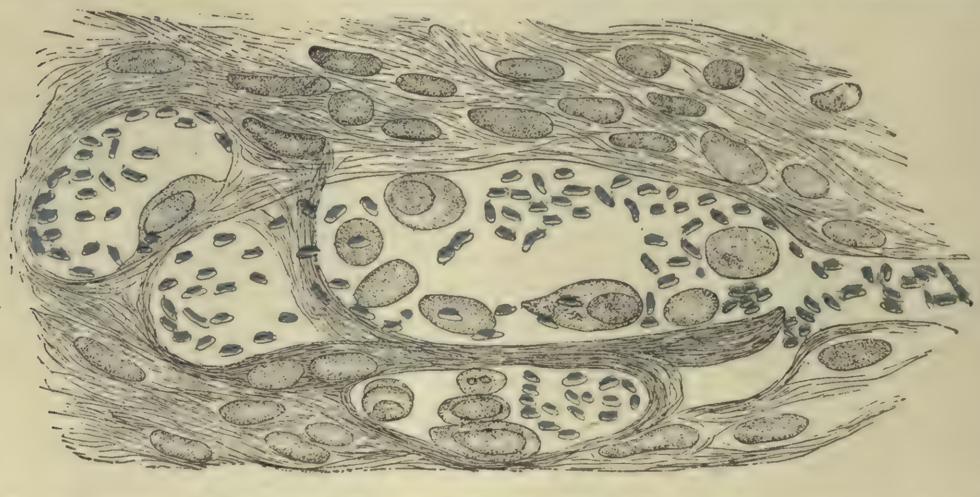

FIG. 126.—Bacillus of rhinoscleroma in lymphatic vessels of the superficial part of tumor. × 1,200. (Cornil and Babes.)

Morphology.—Short bacilli with rounded ends, usually united in pairs, and surrounded by a gelatinous capsule resembling that of Friedländer's bacillus. According to Eisenberg, the bacilli are two to three times as long as broad, and may grow out into filaments.

These bacilli *stain* readily with the aniline colors and by Gram's method. The capsule may be demonstrated by the methods usually employed in staining Friedländer's bacillus, or by the following method which is especially recommended by Alvarez: The excised portions of tissue involved in the disease are placed for twenty-four hours in a one-per-cent solution of osmic acid and then in absolute alcohol. When properly hardened thin sections are made; these are stained in a hot solution of aniline-water-methyl-violet for a few minutes, and then decolorized, by Gram's method, in iodine solution.

Biological Characters.—An *aërobic*, *non-motile*, *non-liquefying* bacillus (facultative anaërobic ?).

In gelatin stab cultures the growth resembles that of Friedländer's bacillus—*i.e.*, a nail-like growth, consisting of densely crowded, opaque colonies along the line of puncture, and a heaped-up, white, glistening mass upon the surface, hemispherical in form and viscous in consistence. Upon *gelatin* plates yellowish-white, spherical colonies are developed within two or three days, which under the microscope are seen to be granular. Upon *potato* a cream-like growth occurs along the line of inoculation, which is white or yellowish-white in color, and in which gas bubbles may be developed. Development is most rapid at a temperature of 35° to 38°, but also occurs at the room temperature.

Pathogenesis.—The etiological relation of this bacillus to the disease with which it is associated has not been established. It is pathogenic for mice and for guinea-pigs, less so for rabbits; in this regard, as in its morphology and growth in various culture media, it bears a close resemblance to Friedländer's bacillus, which is also found not infrequently in the nasal secretions of healthy persons and in those suffering from chronic nasal catarrh or ozæna.

The principal points of difference, as pointed out by Baumgarten, are as follows: The bacillus of rhinoscleroma is usually more decidedly rod-shaped than Friedländer's bacillus, although both may be of so short an oval as to resemble micrococci. The first-mentioned bacillus constantly presents the appearance of being surrounded by a transparent capsule, even in the cultures in artificial media, while Friedländer's bacillus in such media does not usually present this appearance, unless as a result of special treatment. Finally, the bacillus of rhinoscleroma may retain its color, in part at least, when treated by Gram's method, while Friedländer's bacillus is completely decolorized when placed in the iodine solution employed in this method.

Notwithstanding these points of difference, Baumgarten is not entirely satisfied that this bacillus is a distinct species, and several bacteriologists have maintained that it is identical with the bacillus of Friedländer.

XIII.

BACILLI WHICH PRODUCE SEPTICÆMIA IN SUSCEPTIBLE ANIMALS.

When, as a result of accidental (natural) or experimental inoculation, a microörganism is introduced into the body of a susceptible animal which is able to multiply in its blood, producing a general infection, we speak of this general blood infection as a *septicœmia.* When pathogenic microörganisms which are unable to multiply in the blood establish themselves in some particular locality in the animal body which is favorable for their growth, and by the formation of toxic products, which are absorbed, give rise to general symptoms of poisoning, we designate the affection *toxœmia.* As examples of this mode of pathogenic action we may mention diphtheria and tetanus. As a rule, the various forms of septicæmia are quickly fatal, and, as the microörganisms to which they are due multiply in the blood of the infected animal, this fluid possesses infectious properties, and, when inoculated in the smallest quantity into another susceptible animal, reproduces the same morbid phenomena. A typical example of this class of diseases is found in anthrax, to which disease a special section has already been devoted (VIII.). But in this and other forms of septicæmia subcutaneous inoculations do not, as a rule, result in the immediate invasion of the blood by the parasitic microörganism. Often a local inflammatory process of considerable extent is first induced; and in some cases general infection only occurs a short time before the death of the animal, depending, perhaps, upon a previous toxæmia from the absorption of toxic products developed at the seat of local infection. The pathogenic action, then, in acute forms of septicæmia appears to result, not alone from the presence and multiplication of the pathogenic microörganism in the blood, but also from the toxic action of products evolved during its growth.

Some of the pathogenic bacilli of this class now known to bacteriologists have been discovered by studying the infectious diseases induced by them in lower animals among which these diseases prevail naturally—*i.e.*, independently of human interference. Many more are known to us from experiments made in pathological laboratories, in testing by inoculations into animals bacteria obtained from various sources, with reference to their pathogenic power. We include in this group only those bacilli which induce fatal septicæmia in susceptible animals when injected into the circulation or subcutaneously in a comparatively small quantity—*e.g.*, less than half a cubic centimetre of a bouillon culture.

BACILLUS SEPTICÆMIÆ HÆMORRHAGICÆ.

Synonyms.—Bacillus of fowl cholera; Microbe du choléra des poules (Pasteur); Bacillus choleræ gallinarum (Flügge); Bacillus der Hühnercholera; Bacillus of rabbit septicæmia; Bacillus cuniculicida (Flügge); Bacillus der Kaninchenseptikämie (Koch); Bacillus der Rinderseuche (Kitt); Bacillus der Schweineseuche (Löffler and Schütz); Bacillus der Wildseuche (Hueppe); Bacillus der Büffelseuche (Oreste-Armanni); (Bacterium of Davaine's septicæmia ?)

It is now generally admitted by bacteriologists that Koch's bacillus of rabbit septicæmia (1881) is identical with the bacillus ("micrococcus") of fowl cholera previously described by Pasteur (1880). The similar bacilli found in the blood of animals dead from the infectious diseases known in Germany as Wildseuche (Hueppe), Rinderseuche (Kitt), Schweineseuche (Schütz), and Büffelseuche (Oreste-Armanni) appear also to be identical with the bacillus of rabbit septicæmia and fowl cholera. This view is sustained by Hueppe and by Baumgarten, and by the comparative researches of Caneva (1891) and of Bunzl-Federn (1891).

This is evidently a widely distributed pathogenic bacillus; it was obtained by Koch from rabbits inoculated with putrefying flesh infusion, by Gaffky from impure river water, and by Pasteur from the blood of fowls suffering from the infectious disease known in France as *choléra des poules.* It is not infrequently found in putrefying blood, and its presence in the salivary secretions of man has occasionally been demonstrated (Baumgarten).

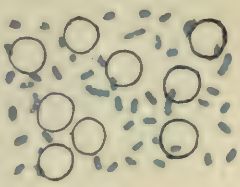
FIG. 127.—Bacillus septicæmiæ hæmorrhagicæ in the blood of a rabbit. × 950. (Baumgarten.)

With reference to the American swine plague described by Salmon and Smith, we are informed by Smith, in his most recent publication upon the subject (*Zeitschrift für Hygiene*, Band x., page 493), that cultures of the German Schweineseuche bacillus, received from the Berlin Hygienic Institute, compared with his cultures from infected swine in this country, agreed in all particulars, except that the former were decidedly more pathogenic for swine and for rabbits.

It appears extremely probable that the form of septicæmia studied by Davaine (1872), which he induced in the first instance by injecting putrid ox blood into rabbits, was due to the same pathogenic bacillus. The writer obtained this bacillus (1887) in Cuba from the blood of rabbits inoculated with liver tissue taken from a yellow-fever cadaver and kept for forty-eight hours in an antiseptic wrapping. The name which we have adopted is that proposed by Hueppe for the form of septicæmia to which it gives rise—"Septikämia hämorrhagica."

Morphology.—Short bacilli with rounded ends, from 0.6 to 0.7 μ in diameter and about 1.4 μ long; sometimes united in pairs, or in chains of three or four elements. In stained preparations the extremities are usually stained, while the central portion of the rod remains unstained. This "end staining" causes the rods to present the appearance of diplococci when examined with a comparatively low power, and some of the earlier observers described the microörganism under consideration as a micrococcus. It is quickly *stained* by the aniline colors usually employed, but loses its color when treated by Gram's method.

Biological Characters.—A *non-motile, aërobic, non-liquefying* bacillus. Does not form spores. Grows in various culture media at the room temperature, but more rapidly at 35° to 37° C.—the lowest temperature at which development occurs is about 13° C. Although this is an aërobic bacillus and a certain amount of oxygen is necessary for its development, it appears to grow better when the amount is somewhat restricted than it does on the surface of nutrient media.

Upon *gelatin plates*, at the end of two or three days, small, white colonies are developed upon or near the surface; these are finely granular and spherical, with a more or less irregular outline, and by transmitted light have a yellowish color; later the central portion of the colonies is of a yellowish-brown color and is surrounded by a transparent peripheral zone. The superficial colonies are commonly smaller than those which develop a little below the surface of the gelatin. In *stab cultures* in nutrient gelatin the growth upon the surface consists of a thin, whitish layer in the vicinity of the point of puncture, having an irregular, jagged outline—sometimes there is no development upon the surface; along the line of puncture the growth consists of rather transparent, discrete or confluent colonies. In *streak cultures* upon nutrient agar, or gelatin, or blood serum the growth is limited to the immediate vicinity of the line of inoculation, and consists of finely granular, semi-transparent colonies, which form a thin, grayish-white layer with irregular, somewhat thickened margins. Upon *potato* no development occurs, as a rule, at the room temperature, but in the incubating oven a rather thin, transparent, grayish-white or yellowish, waxy layer is developed in the course of a few days. According to Bunzl-Federn, the bacillus of fowl cholera and that of rabbit septicæmia grow upon potato, while the bacillus of Wildseuche, Schweineseuche, and Büffelseuche do not. According to Caneva, none of the bacilli of this group grow upon potato. The same author states that the growth *in milk* is scanty and does not produce coagulation, while Bunzl-Federn finds that the bacillus of fowl cholera and of rabbit septicæmia produce coagulation and the others do not. These differences are not, however, considered by the author last named as sufficient to establish the specific difference of the bacilli from these different sources. He looks upon them rather as varieties of the same species. Bunzl-Federn has also ascertained that when cultivated in a peptone solution all of the bacilli of this group, with the exception of that obtained from the so-called Büffelseuche, give the reaction for phenol and for indol—the bacillus of Büffelseuche gives the indol reaction only. Development *in bouillon* is rapid and causes a uniform turbidity of the fluid. Cultures of this bacillus may retain their vitality for three months or more when kept in a moist condition; but the bacillus usually fails to grow after having been kept for a few days in a desiccated condition; according to Hueppe, it may resist desiccation for fourteen days. The *thermal death-point,* as determined by Salmon for the bacillus of fowl cholera, is 56° C., the time of exposure being ten minutes (55° C. with fifteen minutes' exposure—Baumgarten). It is not readily destroyed by putrefaction (Kitt). A solution of mercuric chloride of 1 : 5,000 destroys it in one minute, and a three-per-cent solution of carbolic

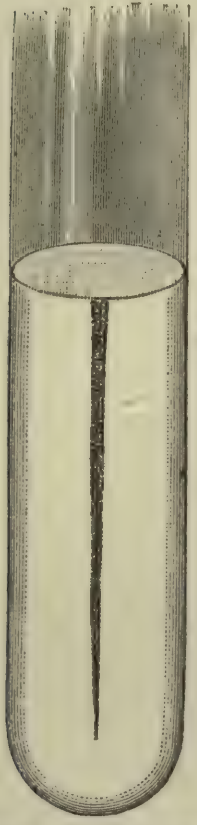

FIG. 128. — Bacillus septicæmiæ hæmorrhagicæ; stick culture in nutrient gelatin, end of four days at 16°-18° C. (Baumgarten.)

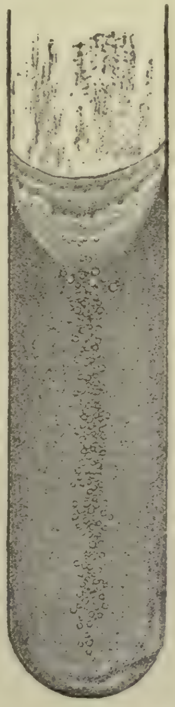

FIG. 129.—Bacillus of Schweineseuche; old stick culture in nutrient gelatin. (Schütz.)

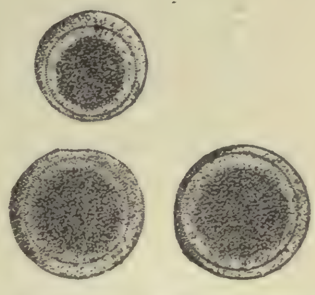

FIG. 130. — Bacillus of swine plague; colonies on gelatin plate, end of seven days. × 60. (Smith.)

acid in six hours (Hueppe). Pasteur (1880) has shown that when cultures of this bacillus (microbe of fowl cholera) in bouillon are kept for some time they gradually lose their pathogenic virulence, and he has ascribed this "attenuation of virulence" to the action of atmospheric oxygen. He also ascertained that the particular degree of virulence manifested by the mother culture after a certain interval could be maintained in successive cultures made at short intervals. He was thus able to cultivate different pathogenic varieties, and to use these in making protective inoculations, by which susceptible animals were preserved from the effects of virulent cultures injected subsequently.

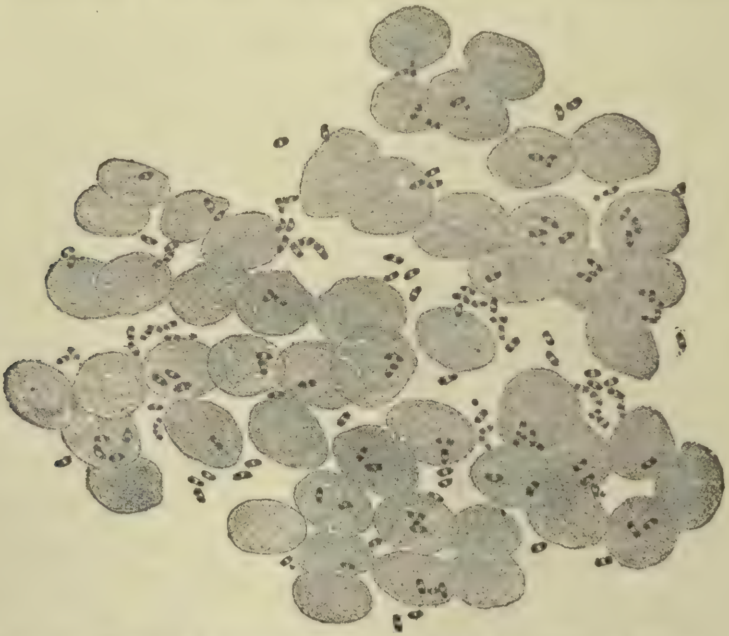

FIG. 131.—Bacillus of Schweineseuche, in blood of rabbit. (Schütz.)

Attenuated cultures recover their virulence when inoculated into very susceptible animals. Thus a culture which would produce a non-fatal and protective attack in a chicken may, according to Pasteur, kill a small bird, like a sparrow; and by successive inoculations from one sparrow to another the original degree of virulence may be restored, so that a minute quantity of a pure culture would be fatal to a chicken.

Pathogenesis.—Pathogenic for chickens, pigeons, pheasants, sparrows, and other small birds, for rabbits and mice, also for swine (Schweineseuche), for cattle (Rinderseuche), and for deer (Wildseuche). (See *supra*, pp. 285–287.)

The researches of Smith and of Moore show that "an attenuated variety of bacteria, belonging to the group of swine-plague bacteria and not distinguishable from them, inhabit the mouth and upper air passages of such domesticated animals as cattle, dogs, and cats" (Smith).

BACILLUS OF CHOLERA IN DUCKS.

Obtained by Cornil and Toupet (1888) from the blood of ducks, in the Jardin d'Acclimation at Paris, which had died of an epidemic disease characterized by diarrhœa, feebleness, and muscular tremors, and which resulted fatally in two or three days.

Morphology.—Does not differ in its morphology from the bacillus of fowl cholera (Bacillus septicæmiæ hæmorrhagicæ); short rods with rounded ends, from 1 to 1.5 μ in length and 0.5 μ broad.

Stains with the usual aniline colors, but not by Gram's method; the ends stain more deeply than the central portion.

Biological Characters.—An *aërobic*, *non-liquefying*, *non-motile* bacillus. Does not form spores. Grows in the usual culture media at the room temperature. In its growth in various media, as well as in its morphology, Cornil and Toupet found this bacillus to correspond with the bacillus of fowl cholera. In *gelatin stab cultures* the growth upon the surface consists of a thin, grayish layer, and along the line of puncture as small, semi-transparent, slightly yellowish, spherical colonies. Upon agar, in the incubating oven, at the end of twelve hours small, lentil-shaped, waxy colonies are formed, which later may have a diameter of three to four millimetres. Upon *potato* circular, yellowish colonies are formed, which become confluent and form a somewhat depressed, pale-yellow layer.

Pathogenesis.—According to Cornil and Toupet, this bacillus is pathogenic for ducks, but not for chickens or pigeons, and only kills rabbits when injected in considerable quantity. Ducks die in from one to three days from subcutaneous injections, or by the ingestion of food to which the bacillus has been added.

BACILLUS OF HOG CHOLERA (Salmon and Smith).

Synonyms.—Bacillus of swine plague (Billings); Bacillus of swine-pest (Selander).

According to Smith, this bacillus was first described by Klein (1884); it was first obtained in pure cultures and its principal characters determined by Salmon and Smith (1885), and has since been studied in cultures and by experimental inoculations by Selander, Billings, Frosch, Welch, Caneva, Bunzl-Federn, and others.

The bacillus is found in the blood and various organs of hogs which have succumbed to the infectious disease known in this country as hog cholera; and also in the contents of the intestine, from which it may be obtained by inoculations into rabbits, but is not easily isolated by the plate method owing to the large number of other bacteria present (Smith).

Morphology.—Short bacilli with rounded ends, 1.2 to 1.5 μ in length and 0.6 to 0.7 μ in breadth; usually united in pairs.

This bacillus is easily *stained* by the aniline colors usually employed, but does not retain its color when treated by Gram's method. When the staining agent is allowed to act for a very short time the ends of the rods may be stained while the central portion remains unstained.

Biological Characters.—An *aërobic* (facultative anaërobic), *non-liquefying, actively motile* bacillus. In many of its characters this bacillus closely resembles the one last described (Bacillus septicæmiæ hæmorrhagicæ), but it is distinguished from it by its active movements, which, according to Smith, may be still observed in cultures which have been kept for weeks or months. Does not form spores. Grows readily in various culture media at the room temperature—more rapidly in the incubating oven. Upon *gelatin plates* colonies are developed in from twenty-four to forty-eight hours. The deep colonies are spherical and homogeneous, and have a brownish color by transmitted light; they seldom exceed one-half millimetre in diameter. The superficial colonies may attain a diameter of two millimetres; they present no distinctive characters. Upon agar plates the colonies may have a diameter of four millimetres; they have a grayish, transparent appearance and a shining surface. In *gelatin stab cultures* small, yellowish-white colonies are developed along the line of inoculation, which may become confluent; upon the surface a thin, pearly layer is developed about the point of inoculation, which may have a diameter of six millimetres or more. Upon *potato* a straw-yellow layer is developed, which later acquires a darker color. In slightly alkaline *bouillon* a slight cloudiness may be observed at the end of twenty-four hours, and at the end of one or two weeks, if not disturbed, a deposit is seen at the bottom of the tube and a thin, broken film may form upon the surface. The development of this bacillus in *milk* produces a direct solution of the casein without previous coagulation; when a solution of litmus has been added to milk

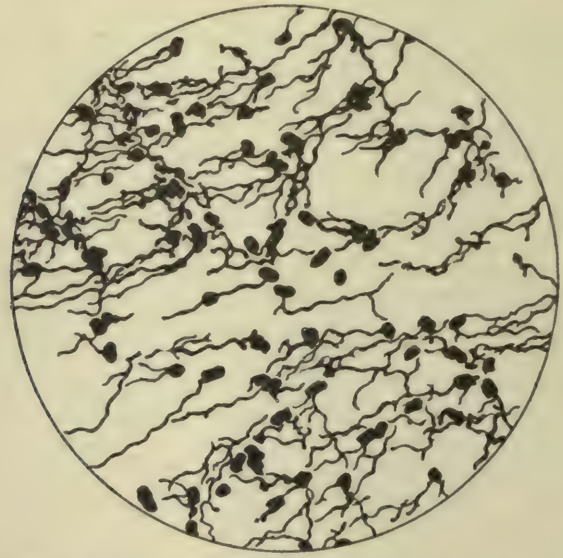

FIG. 132.—Bacillus of hog cholera; stained by Löffler's method to show flagella. × 1,000. From a photomicrograph made at the Army Medical Museum. (Gray.)

it retains its blue color in presence of this bacillus, while the bacillus previously described causes it to change to red. Neither phenol nor indol is produced in solutions containing peptone (Bunzl-Federn) —another distinguishing character from the Bacillus septicæmiæ hæmorrhagicæ. This bacillus may be cultivated in slightly acid media, which after a time acquire an alkaline reaction.

In Smith's experiments this bacillus was found to resist desiccation from nine days to several months, according to the thickness of the layer dried upon the cover glass; bacilli from an agar culture in some experiments failed to grow after seventeen days, and in others still gave cultures after four months. Bouillon cultures are sterilized in four minutes by a temperature of 70° C., in fifteen minutes by 58° C., and in one hour by 54° C. (Smith). Novy has isolated from cultures of the hog-cholera bacillus a toxic basic substance which he calls susotoxin. This was obtained by Brieger's method; it is a yellowish-brown, syrup-like liquid, which, when injected into rats in doses of 0.125 to 0.25 cubic centimetre, causes their death in less than thirty-six hours. He also obtained by precipitation with absolute alcohol, from cultures concentrated in a vacuum at 36° C., a toxalbumin which when dried was in the form of a white powder easily soluble in water. Rats died in three or four hours after receiving subcutaneously a dose of 0.1 to 0.5 gramme.

Pathogenesis.—Pathogenic for swine, rabbits, guinea-pigs, mice, and pigeons.

In certain parts of the United States the disease known as "hog cholera" frequently prevails among swine as a fatal epidemic. It may occur as an acute and quickly fatal septicæmia, or in a more chronic form lasting from two to four weeks or even longer. In the acute form death may occur within twenty-four hours, and hæmorrhagic extravasations are found upon the mucous and serous membranes and in the parenchyma of the lungs, kidneys, and lymphatic glands. The spleen is greatly enlarged, soft, and dark in color. In the chronic form of the disease the most notable changes are found in the alimentary canal. These are most constant and characteristic in the cæcum and colon, which may be studded with spherical, hard, necrotic masses or extensive diphtheritic patches. According to Smith, the hæmorrhagic and necrotic form of the disease may exist at the same time in different animals of the same herd. The bacilli are found in all of the organs, and especially in the spleen, where they are associated in irregular colonies similar to those of the typhoid bacillus. Smith has demonstrated their presence in urine taken from the bladder immediately after the death of the animal, and states that the kidneys are almost always involved, as shown by the presence of albumin and tube casts in the urine.

An extremely minute quantity of a bouillon culture injected beneath the skin of a rabbit causes its death in from seven to twelve days; a larger quantity may produce a fatal result in five days; intravenous injections of very small amounts may be fatal within forty-eight hours. After a subcutaneous injection the animal remains in apparent good health for three or four days, after which it loses its appetite and is indisposed to move; several days before death the temperature is suddenly elevated from 2° to 3° C., and it remains high until the fatal termination. At the autopsy the spleen is found to be enlarged and of a dark-red color; the liver is studded with small, yellowish-white, necrotic foci; the kidneys have undergone parenchymatous changes; the heart is fatty; and the intestinal mucous membrane is more or less marked with hæmorrhagic extravasations. The bacilli are found in all of the organs. In house mice the results of experimental inoculations are similar to those in rabbits. Guinea-pigs succumb when inoculated subcutaneously with one-tenth cubic centimetre; pigeons require a still larger dose—about three-quarters of a cubic centimetre. Swine are killed by the intravenous injection of one to two cubic centimetres of a recent bouillon culture, but, as a rule, do not succumb to subcutaneous injections. Cultures recently obtained from diseased animals are more virulent than those which have been propagated for a considerable time in artificial media.

Smith has described a variety of the hog-cholera bacillus obtained during an epidemic in which the disease was of longer duration—about four weeks—than is usual, and in which there was commonly found at the autopsy a diphtheritic inflammation of the mucous membrane of the stomach. This bacillus differed from the typical form by being somewhat larger and in forming considerably larger colonies in gelatin plates—two or three times as large. It also produced a greater opacity in peptonized bouillon, and in general showed a more vigorous growth in various nutrient media. It differed also in its pathogenic power, as tested upon rabbits, causing death at a later date or not at all; and in fatal cases the swelling of the spleen and necrotic foci in the liver, produced by the first-described species, were absent.

Bang (1892) has obtained a bacillus from infected swine in Denmark which corresponds with the American hog-cholera bacillus. In chronic forms of the disease pneumonia and an extensive diphtheritic process in the intestines occurred as a complication. This was found to be due to another bacillus, called by Bang "vacuole-bacillus." This produced a fatal pleuropneumonia when injected into the lungs in pigs. According to Bang, his "vacuole-bacillus" is without doubt identical with the swine-plague bacillus of Salmon and Smith, and the disease of swine studied by him was a mixed infection. The necrotic changes in the intestine, found in cases running a chronic course, are believed by Bang to be due to still another bacillus—his "necrosis-bacillus." Affanassieff (1892) confirms the results previously obtained by several independent observers as to the identity of the swine-plague bacillus of Salmon and Smith with the Löffler-Schütz bacillus. The only difference observed was a difference in pathogenic virulence—the bacillus from America corresponding with a somewhat attenuated variety of that from Germany.

Welch (1894), as a result of his extended researches, arrives at the following conclusion:

"Our own conclusion as to the bacteria of Schweineseuche and of swine plague is that no difference exists between them as regards morphology, culture behavior, and pathogenic effects on rabbits, mice, and other laboratory animals. Cultures of each occur which are also indistinguishable by inoculation of pigs. The only difference by laboratory experiment which has thus far been brought out is that there occur Schweineseuche bacilli of higher degree of virulence as tested on pigs than any swine-plague bacteria which have hitherto been isolated from pigs in this country. Another point to be considered in this connection is that Schweineseuche occurs as an independent disease in Germany without association with hog cholera, whereas swine plague has not been shown to prevail with the same independence as an epizoötic in this country."

Silberschmidt (1895) arrives at a different conclusion from that reached by Smith, Welch, Bang, and others, He believes that the diseases of swine known as hog cholera, swine plague, and infectious pneumo-enteritis are all due to one and the same bacillus, which, however, varies considerably both in its morphological characters and its pathogenic power. In view of the results previously reached by equally competent bacteriologists, and especially by Smith and by Welch in this country, we are not disposed to accept the view maintained by Silberschmidt.

Smith has described several varieties of the hog-cholera bacillus, and in his account of the "hog-cholera group of bacteria" shows that the *Bacillus enteriditis* of Gärtner and the *Bacillus typhi murium* of Löffler belong to this group. The characters of the different varieties (or species?) belonging to the group are given by Smith in detail (United States Department of Agriculture, Bureau of Animal Industry, Bulletin No. 6, 1894), and the following general statement is made:

"If we attempt to sum up those characters which are to circumscribe the hog-cholera group of bacteria we are at once confronted by the scarcity of common characters. Pathogenesis, though of great importance from the standpoint of pathology, is probably the last character acquired and evidently the most variable and most readily lost. If we base the unity of this group on morphological and biological characters, we are likewise met by variations in size, absence of motility, variations in the appearance of the colonies. There are, however, certain underlying characters, as expressed by the behavior of these bacteria in bouillon containing dextrose, saccharose, and lactose, which I think will serve as a very important group character, differentiating such groups sharply from the colon group. I would therefore suggest that for the present all bacteria whose size approximates that of this group, which do not liquefy gelatin, and whose fermentative properties are the same as those described for this group, should be ranged under it. Future investigations into the biochemical characters of these varieties or sub-species may reveal other differential characters, but the time has not yet come when such laborious work will be undertaken on a sufficiently extensive scale to be of any service in differentiating varieties and sub-species."

Selander in 1890, and Metschnikoff in 1892, have reported a rapid increase in virulence of the bacillus of hog cholera by successive inoculations in rabbits or pigeons. Moore (1894) has shown that this is a mistake, and that the bacteriologists named probably did not experiment with cultures of the hog-cholera bacillus, as they supposed, but that their experiments were made with the bacillus of swine plague—Bacillus septicæmiæ hemorrhagicæ—which when passed through a series of rabbits attains a notable increase in pathogenic virulence.

In a recent article, Klein, of London (1895) says: "The bacillus of English swine plague, which I described in 1884, in Virchow's *Archiv*, as shown by Smith and Welch, is identical with the bacillus of American hog cholera."

BACILLUS OF BELFANTI AND PASCAROLA.

Synonym.—Impftetanusbacillus.

Obtained by Belfanti and Pascarola (1888) from the pus of wounds in an individual who succumbed to tetanus.

Morphology.—Bacilli with rounded ends, sometimes so short as to resemble micrococci; resemble the Bacillus septicæmiæ hæmorrhagicæ (fowl cholera).

Stains with the usual aniline colors and also by Gram's method. The ends are commonly more deeply stained than the central portion.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying, non-motile* bacillus. Spore formation not observed. Grows in the usual culture media at the room temperature. Upon *gelatin plates* yellowish-gray, finely granular, spherical colonies with smooth outlines are developed. In *gelatin stab cultures*, at 18° to 25° C., at the end of twenty-four hours small, spherical colonies are developed along the line of puncture, which are isolated or closely crowded; upon the surface a rather thin, shining, grayish-white, iridescent, circular layer is formed; gas is given off which has not a disagreeable odor. Upon the surface of *agar* elevated, shining, gray colonies develop along the impfstrich, or a gray, shining band is formed which increases in thickness but not in breadth—usually less than one-half centimetre broad. Old cultures give off an acid odor. Upon *blood serum* a thin, white layer is developed along the line of inoculation. Upon *potato* a thin, white, varnish-like layer is formed.

Pathogenesis.—Very pathogenic for rabbits, guinea-pigs, white mice, and sparrows. Not pathogenic for chickens, pigeons, or geese.

BACILLUS OF SWINE PLAGUE, MARSEILLES.

Synonyms.—Bacillus der Schweineseuche, Marseilles (Rietsch and Jobert); Bacillus der Frettchenseuche—ferret disease (Eberth and Schimmelbusch); Bacillus der Amerikanischen Rinderseuche (Caneva); Bacillus of spontaneous rabbit septicæmia (Eberth).

The researches of Caneva and of Bunzl-Federn agree as to the identity of the bacillus obtained by Rietsch and Jobert (1887) from swine attacked with a fatal epidemic disease in Marseilles, and the bacillus found by Eberth and Schimmelbusch (1889) in the blood of ferrets suffering from a fatal form of septicæmia studied by them. The first-named bacteriologist also identifies a bacillus supposed by Billings to be the cause of "Texas fever" in cattle ("Amerikanische Rinderseuche") and the bacillus of swine plague (Billings) with the above. Bunzl-Federn obtained cultures of Billings' swine-plague bacillus at two different times. He identifies the one first received with the bacillus now under consideration, and the other with the bacillus of hog cholera (Salmon).[1]

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[1] The author named says: "With reference to the bacillus of swine plague (Billings), I obtained, as did Caneva, a decided production of acid in the cultures first sent by Billings; but upon testing later cultures received directly from Bil-

Morphology.—Bacilli with rounded ends, about twice as long as broad, and one-third smaller than the bacillus of typhoid fever (Eberth and Schimmelbusch). The bacillus of hog cholera is shorter and more slender than the Marseilles bacillus, and the bacillus of Löffler and Schütz is still smaller (Rietsch and Jobert).

In stained preparations the extremities of the rods are usually deeply stained, while the central portion remains unstained—"polar staining." By Löffler's method of staining the presence of flagella may be demonstrated (Frosch).

Stains readily with the aniline dyes usually employed, but does not retain its color when treated by Gram's method.

Biological Characters.—An *aërobic* (facultative anaërobic), *non-liquefying, actively motile* bacillus. Grows readily at the room temperature, and is distinguished from the bacillus of septicæmia hæmorrhagica by its active movements and more rapid and abundant development in the various culture media usually employed. It is distinguished from the bacillus of hog cholera by producing phenol and indol in solutions containing peptone, by causing coagulation of milk, and by producing an acid reaction in this fluid. Grows in culture media having an acid reaction.

Rietsch and Jobert give the following account of the characters of growth in various culture media, as compared with the bacillus of hog cholera and the bacillus of Schweineseuche (Löffler, Schutz):

Gelatin streak cultures. At the end of twenty-four hours this bacillus had developed considerably, while the growth of the hog-cholera bacillus was scarcely to be discerned with the naked eye, and the bacillus of Schweineseuche did not form a visible growth until the end of forty-eight hours. After several days the bacillus of swine plague (Marseilles) formed an opaque, yellowish-white streak, which, when examined with a low-power lens, had a brown color by transmitted light and a bluish-white color by reflected light. The streak of the Löffler-Schütz bacillus was not so thick and not so opaque, and was made up of small, nearly transparent colonies; the hog-cholera bacillus came between the other two. Upon *blood serum, agar,* and *glycerin-agar* the Marseilles bacillus grew more rapidly than the other two, forming a layer which was opaque and of a white color, with bluish and reddish reflections. Upon *potato* it formed a thick, opaque, yellowish layer, while the growth of the hog-cholera bacillus was much thinner and that of the Löffler-Schütz bacillus scarcely to be seen. In *bouillon* the Löffler-Schütz bacillus, at the end of three days at 37° C., had not produced any perceptible

lings and from other sources, the result was exactly the opposite—viz., a decided production of alkali in milk and identity with the hog-cholera bacillus of Salmon."

cloudiness, while the Marseilles bacillus at the end of twenty-four hours had caused the fluid to be clouded, a film of bacteria had formed upon the surface and a deposit at the bottom of the tube; the hog-cholera bacillus produced a less degree of opacity in the bouillon.

Pathogenesis.—This bacillus is pathogenic for sparrows and other small birds when injected beneath the skin in small amounts, and also for pigeons in a longer time—five to fourteen days. Frosch reports a negative result from subcutaneous injections into rabbits, guinea-pigs, mice, and pigeons, but his cultures appear to have become attenuated, as the recent cultures of Eberth and Schimmelbusch were fatal to pigeons in four out of five experiments. Two rabbits were inoculated subcutaneously by Rietsch and Jobert with half a Pravaz syringeful of a pure culture of the Marseilles bacillus; one of these died on the sixth day and the other survived.

In sparrows, which succumb in from twenty-four to thirty-six hours after receiving a small amount of a pure culture in the breast muscle, the bacillus is present in the blood in large numbers, and a purulent pleuritis and pericarditis is found at the autopsy. In the ferrets from which Eberth and Schimmelbusch obtained their cultures the bacillus was not present in the blood in sufficient numbers to be readily demonstrated by microscopical examination, but it was obtained in pure cultures from the liver, spleen, and lungs. The principal pathological appearances noted were enlargement of the spleen and pneumonia. Caneva reports that the Marseilles bacillus injected into white mice gives rise to an extensive abscess at the point of inoculation, but does not kill adult animals. In a young mouse which succumbed to such an injection the bacilli were not generally distributed in the tissues, but were found as emboli in the smaller capillaries. This bacillus, then, is distinguished from the similar bacilli previously described by its comparatively slight pathogenic power, as well as by its more vigorous growth in culture media, and the other characters heretofore mentioned.

BACILLUS SEPTICUS AGRIGENUS.

Obtained by Nicolaier from soil which had been manured.

Morphology.—Resembles the bacillus of fowl cholera and of rabbit septicæmia, of which it is perhaps a variety, but is usually somewhat longer. It also sometimes shows the end-staining characteristic of Bacillus septicæmiæ hæmorrhagicæ, but not so constantly and not so sharply defined.

Biological Characters.—An *aërobic*, (*non-liquefying ?*), non-motile bacillus. Does not form spores.

In *gelatin plate cultures* spherical, finely granular colonies are developed having a yellowish-brown central portion, which is separated by a dark ring from a grayish-brown marginal zone; later this difference in color disappears and the colonies become more decidedly granular. In stick cultures the growth consists of a thin layer which is not at all characteristic.

Pathogenesis.—Small quantities of a pure culture injected into the ear vein of a rabbit cause its death in from twenty-four to thirty-six hours; pathogenic also for house mice and for field mice. At the autopsy no notable pathological changes are observed. The bacilli are found in blood from the heart and in the capillaries of the various organs, but are not so numerous as in rabbit septicæmia; they show a special inclination to adhere to the margins of the red blood corpuscles.

BACILLUS ERYSIPELATOS SUIS.

Synonyms.—Bacillus of hog erysipelas; Bacillus des Schweinerothlauf (Löffler, Schütz); Bacille du rouget du porc (Pasteur); Bacillus of mouse septicæmia; Bacillus murisepticus (Flügge); Bacillus des Mäuseseptikämie (Koch).

The bacillus of mouse septicæmia, first described by Koch (1878), resembles so closely in its morphology, characters of growth, and pathogenic power the bacillus of Schweinerothlauf of Löffler and Schütz (1885) that they can scarcely be considered as distinct species, although, from slight differences which have been observed, they are perhaps entitled to separate consideration as varieties of the same species. Flügge, Eisenberg, Fränkel, and other authors, while recognizing the fact that the bacilli from the two sources closely resemble each other, apparently do not consider them identical, and describe them separately. Baumgarten, on the other hand, describes them under one heading and considers it highly probable that they are identical, although he also admits slight differences in the morphological characters and growth in culture media. These differences are, however, no greater than we have in artificially produced varieties of other well-known microorganisms, and we think it best to follow Baumgarten in describing them under a single heading.

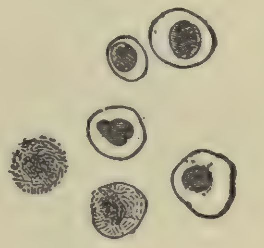

FIG. 133.—Bacillus of mouse septicæmia in leucocytes from blood of mouse. × 700. (Koch.)

Koch first obtained this bacillus by injecting putrefying blood or flesh infusion, during the first days of putrefactive change, beneath the skin of mice. A certain proportion of the animals experimented upon contracted a fatal form of septicæmia, and the bacillus under consideration was found in their blood. The bacillus of Schweinerothlauf was obtained by Löffler and by Schütz from the blood and various organs of swine which had succumbed to the infectious malady known in Germany as rothlauf and in France as rouget.

Morphology.—Extremely minute bacilli, about 1 μ in length and 0.2 μ in diameter. The Schweinerothlauf bacilli are described as somewhat thicker and longer by Flügge, by Fränkel, and by Eisenberg, but Baumgarten states that they are somewhat more slender and on the average shorter than the bacillus of mouse septicæmia. The bacilli are solitary, or in pairs the elements of which are often united at an angle; occasionally a chain of three or four elements may be observed, and in old cultures the bacilli may grow out into short threads which are straight or more or less curved and twisted. Small refractive bodies may sometimes be distinguished in the rods, and these have been supposed by some authors to be spores, but this has not been demonstrated.

This bacillus *stains* readily with the ordinary aniline staining agents and also by Gram's method.

Biological Characters.—A facultative anaërobic, non-liquefying bacillus. According to Schottelius, the rothlauf bacilli are sometimes motile, but Flügge states that other observers have not seen them in active motion. Fränkel says they have the power of voluntary motion. Eisenberg says that the bacillus of mouse septicæmia is motionless, and Fränkel says they "seem to be incapable of voluntary motion." Baumgarten remarks: "Whether the bacilli exhibit voluntary movements has not been determined." Although this bacillus is not strictly anaërobic, it grows better in the absence of oxygen than in its presence. Development occurs in various culture media at the room temperature, but is more rapid in the culture oven. In *gelatin stab cultures* no development occurs upon the surface, but the growth along the line of puncture is very characteristic; this consists of a delicate cloud-like, radiating growth, which extends, in the course of a few days, almost to the walls of the test tube. The rothlauf bacillus does not extend so rapidly through the

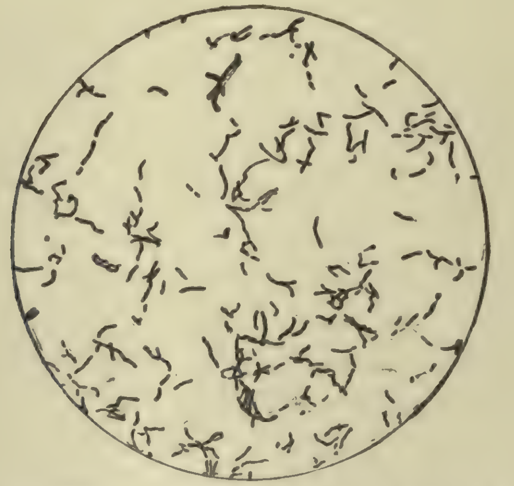

Fig. 134.—Bacillus of rouget, from a pure culture. × 1,000. From a photomicrograph. (Roux.)

gelatin, and the branching, cloud-like growth is not as delicate; Flügge compares it to the brush of bristles used for cleansing test tubes. In old cultures in nutrient gelatin a slight softening of the gelatin occurs along the line of growth, and as a result of evaporation and desiccation a funnel-shaped cavity is formed in the culture medium in the course of two or three weeks. In *gelatin plates* colonies are developed in the course of two or three days in the deeper layers of the gelatin, but not upon the surface; these are nebulous, grayish-blue, radiating masses, which are so delicate as to be scarcely visible without the aid of a lens or a dark background. Under a low power they appear as branching feathery masses, which have been compared by Flügge to the radiating growth of "bone corpuscles." In older cultures they coalesce and cause a nebulous opacity of the whole plate, which has a bluish-gray lustre.

Upon the surface of nutrient agar or blood serum a very scanty development occurs along the line of inoculation. No growth occurs upon potato. In bouillon the bacilli cause a slight cloudiness at the outset, and later a scanty grayish-white deposit upon the bottom of the test tube; no film is formed upon the surface.

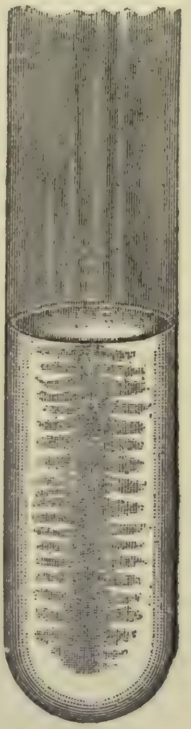

FIG. 135. — Bacillus of mouse septicæmia; culture in nutrient gelatin, end of four days at 18° C. (Baumgarten.)

The thermal death-point of this bacillus, as determined by the writer (1887), is 58° C., the time of exposure being ten minutes. In the experiments of Bolton it was destroyed in two hours by mercuric chloride solution in the proportion of 1:10,000; by carbolic acid and by sulphate of copper in one-per-cent solution. These results are opposed to the view that the minute refractive granules which may sometimes be seen in the interior of the rods are reproductive spores,

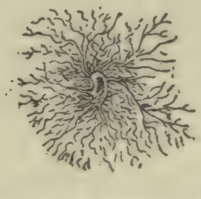

FIG. 136.—Bacillus of mouse septicæmia; single colony in nutrient gelatin. × 80. (Flügge.)

for all known spores have a much greater resisting power to heat and the chemical agents named.

Pathogenesis.—Pathogenic for swine, rabbits, white mice, house mice, pigeons, and sparrows. Field mice, guinea-pigs, and chickens are immune.

Swine may be infected by the ingestion of food containing the rothlauf bacillus, as has been demonstrated by allowing them to eat the intestine of an animal which had recently succumbed to the disease, and also by the subcutaneous injection of pure cultures. The disease usually terminates fatally within three or four days, and sometimes in less than twenty-four hours. It is characterized by fever, debility, loss of appetite, and by the appearance upon the surface of the body of red patches, which gradually extend and become confluent, producing after a time a uniform dark-red or brown color of the entire surface. The discharges from the bowels frequently contain bloody mucus. At the autopsy, in acute cases, the spleen is notably enlarged, and the liver and kidneys are likely to be more or less swollen, as are also the lymphatic glands, especially those of the mesentery; the gastric and intestinal mucous membranes are usually inflamed and spotted with hæmorrhagic extravasations ; the serous membranes also may be inflamed, and the cavities of the pleuræ, pericardium, and peritoneum usually contain more or less fluid. The bacilli are found in the blood vessels throughout the body and are especially numerous in the interior of the leucocytes.

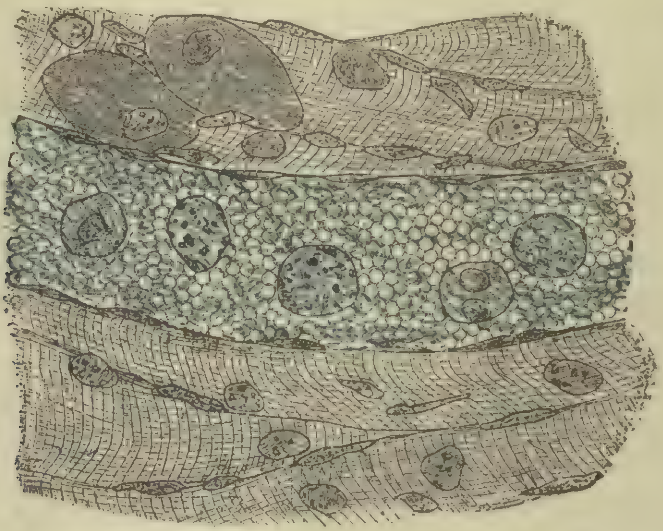

FIG. 137.—Section of diaphragm of a mouse dead from mouse septicæmia, showing bacilli in a capillary blood vessel. (Baumgarten.)

PLATE VI.

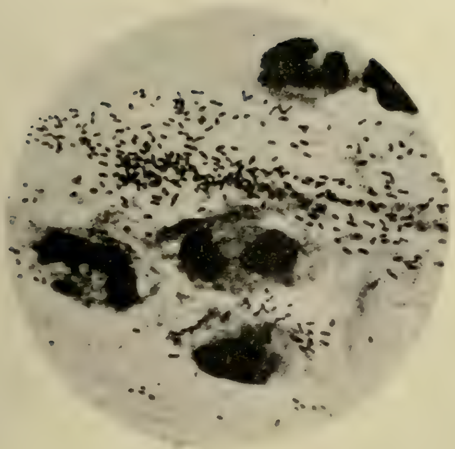

Fig. 1.

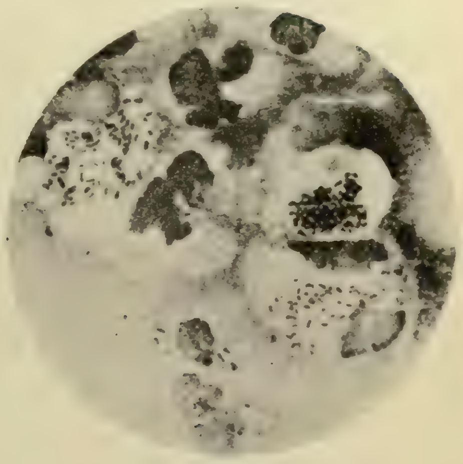

Fig. 2.

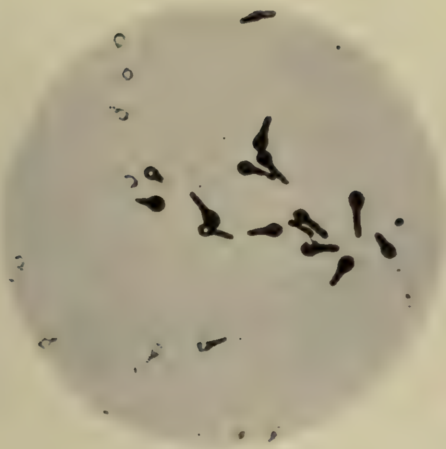

Fig. 3.

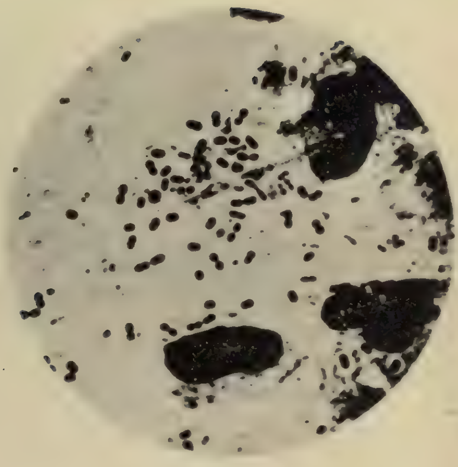

Fig. 4.

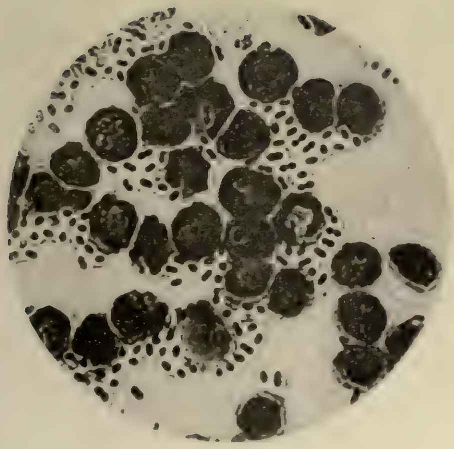

Fig. 5.

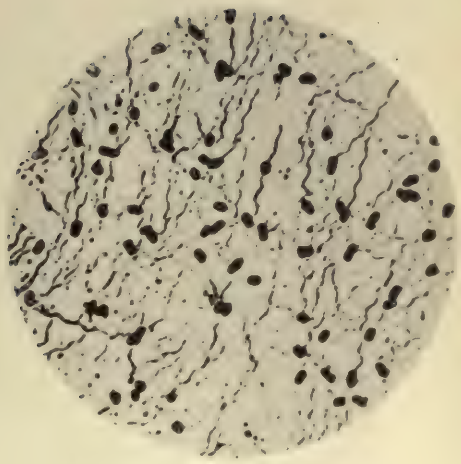

Fig. 6.

Cornevin and Kitt have shown that the contents of the intestine also contain the bacilli in large numbers, and the disease appears to be propagated among swine principally by the contamination of their food with the alvine discharges of diseased animals.

Pigeons are very susceptible to the pathogenic action of this bacillus, and usually die within three or four days after inoculation with a pure culture. Rabbits are not so susceptible, although a certain proportion die from general infection after being inoculated in the ear. The first effect of such an inoculation is to produce an erysipelatous inflammation. When the animal recovers it is subsequently immune.

White mice and house mice are extremely susceptible, but field mice are immune. This remarkable fact was first ascertained by Koch by experiments with his bacillus of mouse septicæmia. House mice which have been inoculated with a minute quantity of a pure culture of the rothlauf, or mouse septicæmia, bacillus, die in from forty to sixty hours. The animal is usually found dead in a sitting position, with its back strongly curved, and for many hours before death it remains quietly sitting in the same position ; the eyes are glued together by a sticky secretion from the conjunctival mucous membrane. At the autopsy the spleen is found to be very much enlarged, and there may be a slight amount of œdema at the point of inoculation.

The bacilli are found in the blood vessels generally, and are very numerous in the interior of the leucocytes, which are sometimes completely filled with them.

BACILLUS COPROGENES PARVUS.

Synonym.—Mäuseseptikämieähnlicher Bacillus (Eisenberg).

Obtained by Bienstock from human fæces.

Morphology.—A very minute bacillus, which is but little longer than it is broad, and might easily be mistaken for a micrococcus.

Biological Characters.—Grows very slowly on nutrient gelatin, forming a scarcely visible film along the line of inoculation, which at the end of several weeks is scarcely one millimetre wide. Is not motile.

Pathogenesis.—In white mice an extensive œdema is developed at the point of inoculation at the end of ten or twelve hours, and the animal dies within thirty-six hours. The bacilli are found in great numbers in the effused serum at the point of inoculation and in comparatively small numbers in the blood. A rabbit inoculated with a pure culture obtained from a mouse died at the end of eight days. The inoculation, which was made in the ear, gave rise to a local erysipelatous inflammation.

BACILLUS CAVICIDA.

Synonym.—Brieger's bacillus. Probably a pathogenic variety of Bacterium coli commune of Escherich.

Obtained by Brieger (1884) from human fæces.

Morphology.—Small bacilli, about twice as long as broad, which closely resemble the colon bacillus of Escherich (Bacterium coli commune).

Biological Characters.—An *aërobic* (facultative anaërobic), *non-liquefying* bacillus.

The growth in gelatin plate cultures is said to be very characteristic, the colonies being "in the form of very beautifully grouped, whitish, concentric rings, which are arranged like the scales upon the back of a turtle" (Eisenberg). The writer has studied cultures of this bacillus brought from the bacteriological laboratories of Germany, side by side with cultures of the Bacterium coli commune of Escherich, and has found no appreciable differences in the colonies in gelatin plates, or in the growth in various culture media. Upon *potato* it grows rapidly in the incubating oven, forming a dirty-yellow, moist layer.

Pathogenesis.—This bacillus, as first obtained by Brieger, was characterized by being very pathogenic for guinea-pigs, which were invariably killed, within seventy-two hours, by the subcutaneous injection of a minute quantity of a pure culture. The bacillus was found in great numbers in the blood of animals which succumbed to an experimental inoculation. The writer's experiments with this bacillus, made in 1889, indicate that its pathogenic power had become attenuated, inasmuch as considerable quantities of a pure culture injected into guinea-pigs did not cause the death of the animals—culture used came originally from Germany. Not pathogenic for rabbits or for mice.

BACILLUS CAVICIDA HAVANIENSIS.

This bacillus was obtained by the writer from the contents of the intestine of a yellow-fever cadaver, in Havana, 1889, through inoculated guinea-pigs.

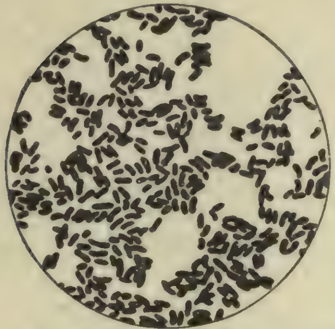

FIG. 138.—Bacillus cavicida Havaniensis; from a potato culture. × 1,000. From a photomicrograph. (Sternberg.)

Morphology.—A bacillus with rounded ends, from two to three μ long and about 0.7 μ broad, frequently united in pairs.

Stains readily with the ordinary aniline colors.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying, actively motile* bacillus.

In *gelatin stab cultures* the growth upon the surface is very scanty and thin, not extending far from the point of puncture; along the line of puncture are developed small, translucent, pearl-like, spherical colonies, which later become opaque and sometimes granular. In gelatin roll tubes, at the end of twenty-four hours at 22° C., the deep colonies are very small spheres, of a pale straw color; later they become opaque, light-brown spheres, or may have a dark central mass surrounded by a transparent zone. The superficial colonies at the end of five days are small, translucent masses of a pale straw color towards the centre, with thin and irregular margins, sometimes with a central light-brown nucleus; at the end of ten days the deep colonies are still quite small, of a brown color, and opaque.

In glycerin-agar roll tubes, at the end of twenty-four hours, the deep colonies are in the form of a biconvex lens, and appear spherical when viewed in face and biconvex when seen from the side; they have a straw color by transmitted light and are bluish-white by reflected light; the superficial colonies are translucent, with a bluish-white lustre.

On *potato*, at 22° C., at the end of forty eight hours there is a thin, dirty-yellow growth of limited extent; at the end of ten days there is a thin, gamboge yellow layer and little masses of the same color; the growth is quite thin, with irregular outlines, and is confined to the vicinity of the impfstrich.

Grows in nutrient agar containing 0.2 per cent of hydrochloric acid. Thermal death point 55° C. Grows in *agua coco* without forming gas, and causes this liquid and bouillon to become slightly translucent—not milky.

Pathogenesis.—Pathogenic for guinea-pigs, less so for rabbits. Guinea-pigs inoculated subcutaneously with a few drops of a pure culture die in ten or twelve hours from general infection. There is usually a considerable effusion of bloody serum in the vicinity of the point of inoculation, and the spleen is more or less enlarged.

BACILLUS CRASSUS SPUTIGENUS.

Obtained by Kreibohm (1886) from the sputum of two individuals, and once in scrapings from the tongue.

Morphology.—Short, thick bacilli, of oblong form, with rounded corners, often bent or twisted—"sausage-shaped." Immediately after division the bacilli are about one-half longer than they are broad, but before dividing again they may attain a length of three to four times the breadth. Irregular forms with swollen ends or uneven contour are frequently seen.

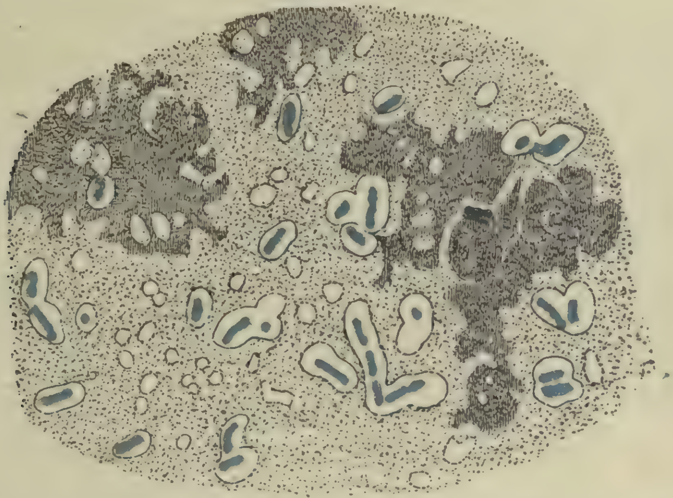

FIG. 139.—Bacillus crassus sputigenus, from blood of mouse. × 700. (Flügge.)

This bacillus is quickly *stained* by the ordinary aniline colors and also by Gram's method.

Biological Characters.—An *aërobic*, *non-liquefying* (non-motile ?) bacillus. Grows in various culture media at the room temperature—more rapidly in the incubating oven. "Appears to form spores at 35° C." (Flügge).

In *gelatin plates*, at the end of thirty-six hours, grayish-white colonies are developed, which soon reach the surface of the gelatin and spread out as round, viscid, grayish white drops, which project considerably above the surface of the culture medium. Under a low magnifying power recent colonies appear as spherical, grayish-brown discs, the surface of which is marked with dark points or lines. The superficial colonies are more transparent, have irregular outlines, and the surface, especially near the margins, is coarsely granular. The development in stab cultures is very rapid and resembles that of Friedländer's bacillus—"nail-shaped" growth. Upon potato the growth is also similar to that of Friedländer's bacillus, and consists of a thick, grayish-white, moist, and shining layer.

Pathogenesis.—Mice inoculated with a small quantity of a pure culture die from acute septicæmia in about forty-eight hours. The bacilli are found in blood from the heart and from the various organs—most numerous in the liver. Rabbits are killed within forty-eight hours by intravenous injection of a small quantity, and the blood contains the bacillus in great numbers. Larger amounts injected into the circulation of rabbits or dogs cause death in a few hours (three to ten), preceded by diarrhœa, and in some instances bloody discharges from the bowels. At the autopsy an acute gastroenteritis is found.

BACILLUS PYOGENES FŒTIDUS.

Obtained by Passet (1885) from an abscess of the anus.

Morphology.—Short bacilli with rounded ends, 1.45 μ long and 0.58 μ broad; usually associated in pairs or in short chains.

Biological Characters.—An *aërobic*, *non-liquefying*, *motile* bacillus. Grows rapidly in the usual culture media at the room temperature. In the interior of the rods, in stained preparations, one or two unstained, spherical places may sometimes be seen, which have been supposed to be spores (?). The independent motion exhibited by this bacillus is not very active. In gelatin plates white colonies are developed at the end of twenty-four hours, which upon the surface spread out as grayish-white plaques, having a diameter sometimes of one centimetre; these are thickest in the centre and of a whitish color; the colonies may become confluent. In *gelatin stab cultures* the growth upon the surface, at the end of twenty-four hours, consists of a thin, grayish white layer with rather thick, irregular margins; along the line of puncture more or less crowded colonies. Upon *potato* the bacillus forms an abundant, shining, pale-brown layer. The cultures give off a disagreeable putrefactive odor.

According to Eisenberg, mice and guinea-pigs are killed in twenty-four hours by injections beneath the skin or into the cavity of the abdomen, and numerous bacilli are found in the blood.

PROTEUS HOMINIS CAPSULATUS.

Obtained by Bordoni-Uffreduzzi (1887) from two cadavers presenting the pathological appearances of the so-called "Hadernkrankheit."

Morphology.—Bacilli, varying considerably in dimensions; somewhat thicker than the anthrax bacillus; often swollen in the middle or at the extremities; more or less curved; isolated, united in pairs or in long filaments; in stained preparations from agar cultures or from blood the bacilli are surrounded by a "capsule."

Stains with the usual aniline colors and also by Gram's method.

Biological Characters.—An *aërobic* (facultative anaërobic ?), *non-liquefying*, *non-motile* bacillus. Formation of spores not observed. Grows in the usual culture media at the room temperature. At a temperature of 15° to 17° C. long filaments are formed, in which the bacilli are surrounded with a capsule; at 22° to 24° C. the bacilli are for the most part isolated, but few filaments being formed; at 32° to 37° C. the bacilli are so short as to resemble micrococci; development ceases at a temperature of 8° and is very slow at 15° C.

This bacillus grows as well in an acid medium as in one which is slightly alkaline. In *gelatin plates*, at the end of eighteen to twenty-four hours, colonies are formed which under a low power are seen to be spherical and to contain a quantity of shining granules; the following day, at a temperature of 15° to 17° C., the colonies may be as large as a pin's head and still remain spherical or slightly oval, but the outline is no longer so uniform, and between the shining points in the interior a confused network may be seen; as the colony becomes larger it is raised above the surface of the gelatin, becomes opaque, and has a pearly lustre like that of Friedländer's bacillus. In *gelatin stab cultures* the growth resembles that of Friedländer's bacillus—"nail-shaped growth." Upon the surface of *nutrient agar* a rapidly extending, semi-transparent layer is formed. Upon *potato*, at 15° to 17° C., at the end of twenty-four hours transparent drops are seen in the vicinity of the point of inoculation, and later a moist, shining, colorless layer, of tough consistence, is formed, which gradually extends over the surface. The growth upon *blood serum* resembles that upon nutrient agar, and the blood serum is not liquefied. In liquid blood serum or in bouillon the bacilli are isolated—not in filaments; they cause a clouding of the liquid, and an abundant deposit accumulates at the bottom of the tube, while a film of bacilli forms upon the surface. The cultures never give off a putrefactive odor.

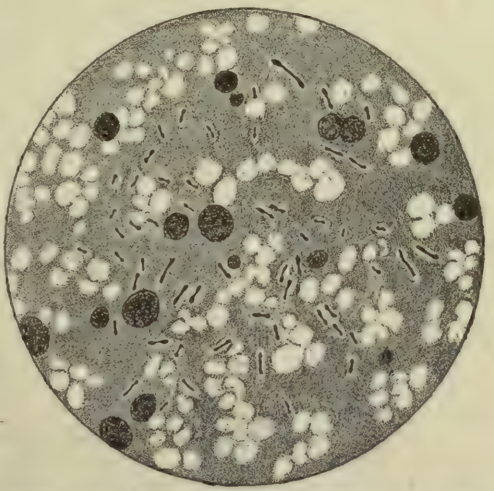

Fig. 140.—Proteus hominis capsulatus, from liver of mouse. × 1,000. (Bordoni-Uffreduzzi.)

Pathogenesis.—Pathogenic for dogs and for mice, less so for rabbits and for guinea-pigs. Agar cultures grown in the incubating oven at 32° to 37 C. are more pathogenic than cultures in gelatin at the room temperature. A small quantity of a recent culture injected subcutaneously in mice causes their death in from one to four days, according to the quantity and age of the culture; the recent cultures are most virulent. When the animal lives more than twenty-four hours it has a mucous diarrhœa. At the autopsy the spleen is found to be much enlarged and dark in color; the lymphatic glands are also swollen and hæmorrhagic, the liver and kidneys hyperæmic; in the vicinity of the point of inoculation is a subcutaneous œdema of jelly-like appearance and numerous punctiform hæmorrhages are seen. The bacillus is found in great numbers in the effused serum from the subcutaneous tissues, in the blood, the contents of the intestine, and in the parenchyma of the various organs. When examined at once the bacilli in the subcutaneous œdema and in the lymphatic glands are usually quite short, and even spherical, while in the blood they are somewhat longer and may appear as short filaments with swollen ends, surrounded by a capsule. When the examination is made some time after the death of the animal longer filaments are quite numerous. Rabbits and guinea-pigs are killed by the intravenous injection of comparatively small amounts of a recent culture, but quite large doses are required to produce a fatal result when the injection is made beneath the skin. From two to three cubic centimetres of a recent culture injected into the circulation of a dog give rise to symptoms of toxæmia, and the animal usually dies on the second day. At the autopsy the abdominal organs are found to be hyperæmic, the mucous membrane of the intestine swollen, red in color, and covered with bloody mucus. The bacillus is found in the blood and in the various organs. When smaller doses are injected into a vein (a few drops) the animal, after a few hours, has a mucous diarrhœa and vomiting, or efforts to vomit. Death usually occurs at the end of two or three days. At the autopsy the spleen is found to be normal, the other organs slightly hyperæmic, and the intestinal mucous membrane in a state of catarrhal inflammation. The bacilli are found in the blood and in the various organs in considerable numbers.

PROTEUS CAPSULATUS SEPTICUS.

Obtained by Banti (1888) from a case of "acute hæmorrhagic infection."

According to Banti, this is possibly identical with the preceding species—Proteus hominis capsulatus—but in some respects more nearly resembles Friedländer's bacillus.

BACILLUS ENTERITIDIS.

Obtained by Gärtner (1888) from the tissues of a cow which was killed in consequence of an attack characterized by a mucous diarrhœa, and also from the spleen of a man who died twelve hours after eating the flesh of this animal.

Morphology.—Short bacilli, about twice as long as broad, frequently united in pairs; chains of four to six elements are sometimes seen.

Stains with the usual aniline colors, and presents the peculiarity of staining deeply at one end while the remainder of the rod is but slightly stained. When two bacilli are united the deeply stained ends are in apposition.

Biological Characters.—An *aërobic*, *non-liquefying*, *motile* bacillus. Spore formation not determined. Grows in the usual culture media at the room temperature. Upon *gelatin plates* pale-gray, superficial colonies are formed at the end of twenty-four hours; under a low power these are seen to be coarsely granular and transparent; the central portion usually presents a greenish color; deep colonies are spherical, indistinctly granular, and of a brownish color; in older colonies a marginal transparent zone is seen which appears to be made up of minute fragments of glass of a pale-brown color. In *gelatin stab cultures* but slight development occurs along the line of puncture; upon the surface a thick, grayish-white layer is formed, which after a time becomes very much wrinkled. Upon the surface of *agar*, at 37° C., at the end of eighteen to twenty hours a grayish-yellow layer has formed. Upon *potato* a moist, shining, yellowish-gray layer is developed. The growth upon *blood serum* is rapid in the form of a gray layer along the line of inoculation.

Pathogenesis.—White mice and house mice usually die in from one to three days when fed with a pure culture of this bacillus. Rabbits and guinea-pigs die in from two to five days from subcutaneous injections—less pathogenic for pigeons and canary birds. Dogs, cats, chickens, and sparrows are immune. A goat died in twenty hours after receiving an intravenous injection of two cubic centimetres of a culture in blood serum. The principal pathological appearance consists in an intense inflammation of the intestinal mucous membrane. The bacilli are found in blood from the heart and also in the contents of the stomach.

BACILLUS OF GROUSE DISEASE.

Obtained by Klein (1889) from the lungs and liver of grouse which had succumbed to an epidemic disease.

Morphology.—Bacilli with rounded ends, from 0.8 to 1.6 μ long; may also be seen as spherical or oval cells 0.6 μ long and 0.4 μ thick; solitary, in pairs, or in chains of three to four elements.

Stains best with Weigert's solution of methylene blue in aniline water.

Biological Characters.—An *aërobic*, *non-liquefying*, *non-motile* bacillus. Spore formation not observed. Grows in the usual culture media at the room temperature—better in the incubating oven. Upon *gelatin plates*, at 20° C., at the end of twenty-four hours small, angular, transparent scales may be seen upon the surface with a low-power lens; at the end of three or four days these form flat, more or less irregular, shining, gray colonies, with thin and often dentate margins; these colonies may become confluent and form a dry, scaly layer which by reflected light has a peculiar, fatty lustre In *gelatin stab cultures* the superficial growth is in the form of a transparent, dry, grayish layer with dentate margins, not more than three to five millimetres in diameter. Upon *agar*, at 36° to 37° C., a thin, whitish-gray, dry layer is formed.

Pathogenesis.—Pathogenic for mice, for guinea-pigs, for linnets, and for green-finches; less so for sparrows. Chickens, pigeons, and rabbits, according to Klein, are immune. Of eight mice inoculated subcutaneously with one or two drops of a bouillon culture, six died within forty-eight hours and two recovered. Out of eight guinea-pigs inoculated in the same way four died in forty-eight hours and two recovered. At the autopsy the lungs and liver were found to be hyperæmic, the spleen not enlarged. The bacilli were present in large numbers in blood from the heart and in the lungs.

BACILLUS GALLINARUM.

Obtained by Klein (1889) from the blood of chickens which succumbed to an epidemic disease resembling "fowl cholera." The bacillus is believed by Klein not to be identical with Pasteur's bacillus of fowl cholera, and is said not to be pathogenic for rabbits, which would seem to differentiate it from this bacillus (Bacillus septicæmiæ hæmorrhagicæ).

Morphology.—Bacilli with rounded ends, from 0.8 to 2 μ long and 0.3 to 0.4 μ thick; often in pairs.

Stains with the usual aniline colors.

Biological Characters.—An *aërobic*, *non-liquefying*, *non-motile* bacillus. Does not form spores. Grows in the usual culture media at the room temperature—better in the incubating oven. Upon *gelatin plates* forms grayish-white, superficial colonies, which later present the appearance of flat, homogeneous, whitish discs with thin edges and irregular margins, and by transmitted light have a brownish color. The deep colonies are small and spherical, and have a brownish color by transmitted light. In *gelatin stab cultures* a thin, gray layer with irregular margins and of limited extent forms upon the surface, and a scanty growth occurs along the line of puncture in the form of a grayish-white line. Upon the surface of *agar*, at 37° C., a thin, gray layer with irregular margins has developed at the end of twenty-four hours; later this extends over the entire surface as a thin, grayish-white layer. No growth occurs upon *potato* at 37° C. In *bouillon*, at 37° C., development occurs, with clouding of the bouillon, within twenty-four hours; later a deposit consisting of bacilli is seen at the bottom of the tube, but no film forms upon the surface.

Pathogenesis.—Chickens inoculated subcutaneously with a pure culture die in from twenty-four hours to eight or nine days. Pigeons and rabbits are immune.

BACILLUS CAPSULATUS.

Obtained by Pfeiffer (1889) from the blood of a guinea-pig which died spontaneously.

Morphology.—Thick bacilli with rounded ends, usually two or three times as long as broad; often united in chains of two or three elements; may grow out into homogeneous filaments. Stained preparations show the bacilli to be enveloped in an oval capsule which may be considerably broader than the bacilli themselves—two to five times as broad; where several bacilli are united they are surrounded by a single capsular envelope.

Stains with the usual aniline colors, but not by Gram's method. In preparations which are deeply stained with hot fuchsin or gentian violet solution the capsule is so deeply stained that the bacillus is hidden; by careful treatment with a weak solution of acetic acid the capsule may be differentiated as a pale-red or violet envelope surrounding the deeply stained bacilli.

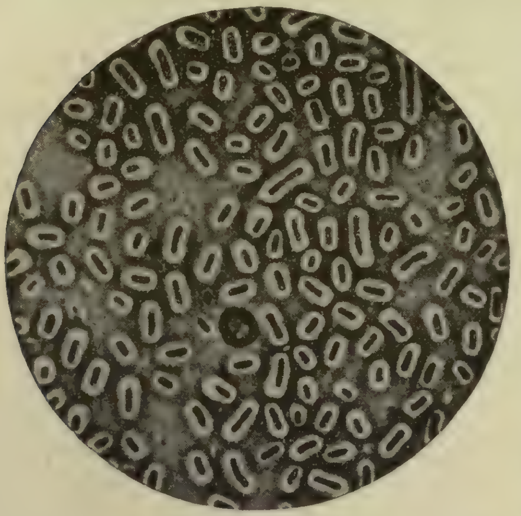

Fig 141.—Bacillus capsulatus, from peritoneal exudate of an inoculated guinea-pig. × 1,000. From a photomicrograph. (Pfeiffer.)

Biological Characters.—An *aerobic* and *facultative anaërobic, non-liquefying, non-motile* bacillus. Spore formation not observed. Grows in the usual culture media at the room temperature. The cultures in agar or upon potato are very viscid and draw out into long threads when touched with the platinum needle; the blood of an animal killed by inoculation with this bacillus has the same viscid character. Upon *gelatin plates* minute colonies are first visible at the end of twenty-four to thirty-six hours; later the deep colonies are white, oval masses the size of a pin's head; the superficial colonies attain the size of a lentil, and are flattened, hemispherical masses with a porcelain-white color. In *gelatin stab cultures* growth occurs to the bottom of the line of puncture, and on the surface a shining white, circular, arched mass forms around the point of puncture, resembling the growth of Friedländer's bacillus. Upon the surface of *agar*, at 37° C, at the end of twenty-four hours a thick, soft layer of a pure white color is formed, which is very viscid and resembles the growth of Micrococcus tetragenus upon the same medium. Upon *potato* an abundant and viscid, shining, yellowish-white layer is quickly developed.

Pathogenesis.—Pathogenic for white mice and for house mice, which die at the end of two or three days after being inoculated at the root of the tail with a small quantity of a pure culture. Inoculation from mouse to mouse increases the virulence of the cultures. At the autopsy the superficial veins are distended with blood, the inguinal glands enlarged, the spleen considerably enlarged, the liver and kidneys hyperæmic, the intestine pale, the heart distended with blood, which usually is very viscid and is drawn out into threads when touched with the platinum needle. The bacilli are found in the blood and in all of the organs, in the contents of the peritoneum and pleuræ, and in the exudate in the vicinity of the point of inoculation. Pathogenic also for guinea pigs and for pigeons; guinea-pigs are infallibly killed within thirty-six hours by the injection of a single drop of a bouillon culture, twenty-four hours old, into the cavity of the abdomen; the blood contains the bacillus in enormous numbers, as does the viscid fluid found in the peritoneal cavity. Rabbits do not succumb to intraperitoneal or subcutaneous inoculations, but are killed by the intravenous injection of one cubic centimetre of a recent bouillon culture. Putrefactive changes occur very quickly in animals killed by inoculation with this bacillus.

BACILLUS HYDROPHILUS FUSCUS.

Obtained by Sanarelli (1891) from the lymph of frogs suffering from a fatal infectious disease.

Morphology.—Bacilli with rounded ends, usually from 1 to 3 μ in length; often short oval; may grow out into filaments of 12 to 20 μ in length.

Biological Characters.—An *aërobic, liquefying, motile* bacillus. Grows in the usual culture media at the room temperature. In *gelatin stab cul-*

tures, at 18° to 20° C., liquefaction has already commenced along the line of puncture at the end of twelve hours, and at the end of thirty-six to forty-eight hours half of the gelatin is liquefied in funnel shape; on the third or fourth day the gelatin is completely liquefied, and a thick, white, flocculent deposit is seen at bottom of the tube. In *glycerin-agar*, at 37° C., a slight, bluish, diffuse fluorescence is seen upon the surface at the end of twelve hours, and soon after a luxuriant growth, which soon covers the entire surface, is developed; at the end of twenty-four to thirty-six hours large gas bubbles begin to form in the agar; gradually the fluorescence disappears, the surface growth becomes thicker and has a dirty-gray color which changes later to brownish. *Blood serum* is a favorable medium and is rapidly liquefied by this bacillus. Upon *potato* the growth is most characteristic. At the end of twelve hours a thin, straw-yellow layer is developed along the impfstrich; this gradually becomes yellow, and at the end of four to five days has a brown color, resembling that of the glanders bacillus upon potato.

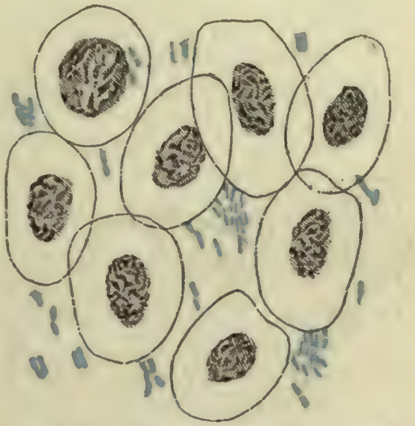

FIG. 142.—Bacillus hydrophilus fuscus, in blood of triton. (Sanarelli.)

Pathogenesis.—Pathogenic for frogs, toads, lizards, and oth "cold-blooded" animals; also for guinea-pigs, rabbits, dogs, cats, mice, chickens, and pigeons. When a few drops of a bouillon culture are injected into the muscles of the thigh, swelling and redness at the point of inoculation are quickly developed, and death usually occurs in eight to ten hours. The bacilli are found in great numbers in the blood and in all of the organs. Guinea pigs die from general infection within twelve hours after receiving a subcutaneous injection of a small amount of a pure culture; the spleen is enlarged and the liver and spleen hyperæmic; an extensive inflammatory œdema in the vicinity of the inoculation wound is frequently observed; the bacilli are very numerous in the blood and in all the organs. Rabbits die in five to six hours from an intravenous injection. Adult dogs are immune, but new-born dogs (three to four days old) die infallibly, after receiving a subcutaneous injection of a small quantity of a pure culture, in twelve to thirty-six hours. Young cats also succumb to similar inoculations. Chickens and pigeons die within five to seven hours after receiving an intravenous injection, but resist subcutaneous injections.

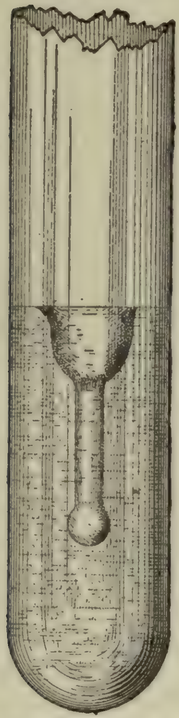

FIG. 143.—Bacillus hydrophilus fuscus; culture in nutrient gelatin, end of sixteen hours. (Sanarelli.)

BACILLUS TENUIS SPUTIGENUS.

Obtained by Pansini (1890) from sputum.

Morphology. Short bacilli, usually in pairs and surrounded by a capsule.

Stains by Gram's method.

Biological Characters.—An *aërobic*, *non-liquefying*, *non-motile* bacillus. Grows in nutrient gelatin at the room temperature. Develops abundantly on *potato*. Coagulates *milk* and produces an acid reaction in this medium.

Pathogenesis.—Pathogenic for rabbits and white rats; not for guinea-pigs or for white mice (in small doses).

BACILLUS OF LASER.

Obtained by Laser (1892) from mice which succumbed to an epidemic disease in Fränkel's laboratory at Königsberg.

In its characters this bacillus closely resembles the bacillus of swine plague, and is perhaps identical with it.

Morphology.—A small bacillus, with rounded ends, about twice as long as broad. Has flagella both at the extremities and sides.

Stains by the usual aniline colors and also by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying, actively motile* bacillus. Spore formation not observed. Grows either in the incubating oven or at the room temperature. Thermal death-point 65° to 70° C.—ten minutes' exposure. Upon *gelatin plates*, at the end of two days, the deep colonies are spherical, finely granular, and brownish in color; the superficial are transparent, finely granular, and leaf-like. In *gelatin stab cultures* growth occurs along the entire line of puncture as well as upon the surface. At the end of three days a considerable evolution of gas is usually observed. In *agar* an abundant development is seen at the end of twenty-four hours in the incubating oven; upon the surface a grayish-white, shining layer with dentate margins is formed along the track of the needle. In *bouillon*, at 37° C., development is abundant and rapid; a thin film is formed on the surface at the end of the second day. Upon *potato* a brownish layer is formed at the end of twenty-four hours. In *milk* an acid reaction is produced.

Pathogenesis.—Pathogenic for field mice, guinea-pigs, rabbits, and pigeons. The bacillus is found in the blood and various organs of infected mice. The spleen is found to be greatly enlarged.

BACILLUS TYPHI MURIUM (Löffler).

Obtained by Löffler (1889) from mice which died in his laboratory from an epidemic disease due to this bacillus.

Morphology.—Short bacilli, resembling the bacillus of diphtheria in pigeons, and varying considerably in dimensions—like the bacillus of typhoid fever; grows out into flexible filaments.

Stains with the aniline colors—best with Löffler's solution of methylene blue.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying, motile* bacillus. Spore formation not determined. Has flagella around the periphery of the cells, like those of the typhoid bacillus, and exhibits similar active movements. In *gelatin stab cultures*, at the room temperature, growth occurs upon the surface, at the end of forty-eight hours, in the form of a flat, grayish-white, round, semi-transparent mass the size of a pin's head; later the surface colony increases in extent and has more or less irregular margins. In *gelatin plate cultures* the deep colonies are at first round, slightly granular, transparent, and grayish; later they are of a yellowish-brown color and decidedly granular. The superficial colonies are very granular and marked by delicate lines—similar to colonies of the typhoid bacillus. Upon *agar* a grayish-white layer is developed which is not at all characteristic. Upon *potato* a rather thin, whitish layer is formed, and around this the potato acquires a dirty bluish-gray color. In *milk* an abundant development occurs, and a decidedly acid reaction is produced without causing any perceptible change in the appearance of the fluid.

Pathogenesis.—Pathogenic for white mice, which die in from one to two weeks after infection; also to field mice, which succumb to subcutaneous injections of a pure culture, and also, in from eight to twelve days, when fed upon potato cultures or bread moistened with a small quantity of a bouillon culture. Löffler believes that this bacillus may be used for the destruction of field mice in grain fields, inasmuch as they invariably die after ingesting food which has been contaminated with it, and also from eating the bodies of other mice which have died as a result of infection. House mice are also susceptible. Rabbits, guinea pigs, pigeons, and chickens were found by Löffler not to be susceptible to infection by feeding.

BACILLUS OF CAZAL AND VAILLARD.

Obtained by Cazal and Vaillard (1891) from cheesy nodules upon the peritoneum and in the pancreas of an individual who died in the hospital at Val de Grace.

Morphology.—Bacilli with rounded ends, but little longer than they are broad; solitary, in pairs, or in chains of ten to fifteen or more elements.

Stains with the usual aniline colors, but not by Gram's method; the extremities of the rods are more deeply stained than the central portion—"polar staining."

Biological Characters.—An *aërobic* and *facultative anaërobic*, *liquefying*, *motile* bacillus. Does not form spores. Grows in the usual culture media at the room temperature—more rapidly in the incubating oven at 37° C. In *gelatin stab cultures*, at the end of twenty-four hours, a series of punctiform, white colonies is developed along the line of puncture; upon the surface development is more abundant, and at the end of forty-eight hours liquefaction commences; this progresses slowly from above downward, and a white, flocculent deposit accumulates at the bottom of the liquefied gelatin. Upon the surface of *agar*, at the end of twenty-four hours at 37° C., a moist, transparent, opalescent layer is developed, which rapidly extends over the entire surface; later this layer becomes somewhat thicker, whitish, and cream-like in consistence, without losing its transparency. Upon *potato* a thick, prominent, moist, and slightly viscid layer is developed, which at first has a pale-yellow and later a yellowish-brown color. In *bouillon* development is abundant, producing a milky opacity of the liquid; a thick, flocculent deposit accumulates at the bottom of the tube; the reaction of the culture liquid becomes very alkaline. All of the cultures give off a peculiar odor, slightly ammoniacal and resembling that of putrid urine. The cultures retain their vitality for several months—in a closed tube for more than a year. The thermal death-point is 60° C. with fifteen minutes' exposure.

Pathogenesis.—Pathogenic for rabbits and mice, but not for guinea-pigs. In mice death occurs from general infection, at the end of forty-eight to sixty hours, from the subcutaneous injection of one eighth cubic centimetre of a recent bouillon culture. In rabbits injection of one cubic centimetre into the circulation causes the death of the animal in thirty-six to fifty hours. The symptoms induced are a fœtid diarrhœa and paralysis of the extremities. When smaller doses are injected (0.5 cubic centimetre) a chronic malady is developed, characterized at the outset by diarrhœa and emaciation, then by the development of tumors which resemble those found in the man from whom the cultures were first obtained. These tumors are for the most part located in the subcutaneous connective tissue; after a time they attain the size of a chestnut and ulcerate, allowing the escape of a semi-fluid, purulent material. The animals usually recover. Similar tumors are developed as a result of subcutaneous injections of one to three cubic centimetres of a recent bouillon culture.

BACILLUS OF BABES AND OPRESCU.

Obtained by Babes and Oprescu (1891) from a case of septicæmia hæmorrhagica presenting some resemblance to exanthematic typhus.

Morphology.—In agar cultures the bacilli are from 0.4 to 0.5 μ thick, and are frequently united in pairs; associated with these rod-shaped bacteria are forms which are of a short oval. In gelatin cultures oval forms are more numerous; they have a diameter of 0.3 to 0.4 μ, and often appear to be surrounded by a capsule. In fresh cultures the bacilli are often in form of a figure 8, and are only stained at the point of contact of the two segments. In potato cultures they are sometimes elongated and swollen at one extremity.

Stains with the usual aniline colors and by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic*, *non-liquefying*, *actively motile* bacillus. Spore formation not observed. Grows in the usual culture media at the room temperature—more rapidly at 37° C. In *gelatin stab cultures* yellowish-white colonies are developed along the line of puncture; at the bottom these may have a diameter of one to two millimetres, and they have a brown color. Upon the surface an irregular, lobulated, whitish, translucent, paraffin like layer is developed. At the end of eight days the surface growth consists of large, confluent, transparent plaques, with irregular outlines and crenated, elevated margins; along the line of puncture large, separate, lenticular or spherical colonies are seen; these have a brownish-white color. At the end of two months the surface growth is concentric and still more transparent, while the colonies near the surface have become almost brown. Upon the *surface of agar*, at 37° C., a narrow band is developed along the line of inoculation; above, this is composed of transparent, shining, flat, round colonies having a diameter of one millimetre or more; below, the colonies are confluent and form a transparent, whitish layer. In glycerin-agar development is still more abundant, and may already be perceived at the end of twelve hours. Crystals are seen below the surface in agar cultures and about the superficial colonies in gelatin. Upon *potato* a uniform, thin, grayish, very transparent layer is developed, which sometimes has a brownish-gray tint. At the end of a few days the potato acquires a brownish color. In *bouillon* cloudiness of the medium is apparent at the end of ten hours; twenty-four hours later a whitish precipitate is seen at the bottom of the tube, which is more abundant when the culture medium contains glucose; later a thin pellicle is seen upon the surface and the bouillon acquires a yellowish color.

Pathogenesis.—Recent cultures are pathogenic for rabbits, guinea-pigs, pigeons, and mice, which die from general infection in from two to four days. Old cultures are less virulent.

BACILLUS OF LUCET.

Obtained by Lucet (1891) from chickens and turkeys suffering from an infectious form of septicæmia characterized by dysenteric discharges—"Dysenterie epizootique des poules et des dindes."

Resembles Bacillus gallinarum of Klein, and is perhaps identical with this microörganism.

Morphology.—Short bacilli, from 1.2 to 1.8 μ long, usually in pairs.

Stains with the usual aniline colors, but not by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic*, *non-liquefying*, *non-motile* bacillus. Spore formation not observed. Grows slowly in the usual culture media at the room temperature—more rapidly at 37° C.

In *gelatin plates* small, shining, moist, white, circular colonies are developed, which look like little drops of wax; later these increase in size, and especially in thickness, forming hemispherical masses. In *gelatin stab cultures* grayish, punctiform colonies are developed along the line of puncture, and upon the surface a circular, prominent, whitish plaque. Streak cultures upon the surface of gelatin are in the form of a dirty-white or grayish-white, moist streak, with regular margins, limited to the line of inoculation, but increasing in thickness until it breaks loose and slips down the oblique surface of the culture medium. The deposit which collects in this way acquires, as it becomes old, in the deepest portion a reddish color. Upon *agar* it forms a thick, yellowish-white, mucus-like layer with straight or slightly dentate margins. In *bouillon* it produces a decided clouding of the liquid, and an abundant grayish, pulverulent sediment accumulates at the bottom of the tube; the bouillon after a time becomes transparent above this sediment and

PLATE VII.

BACILLUS OF GLANDERS.

Fig. 1.—Bacillus mallei from the liver of a field mouse, cover-glass preparation. (Löffler.)

Fig. 2.—Bacillus mallei from a recent culture upon blood serum. (Löffler.)

Fig. 3.—Bacillus mallei in section of spleen of a field mouse dead from glanders. (Löffler.)

Fig. 4.—Culture of glanders bacillus upon cooked potato. (Löffler.)

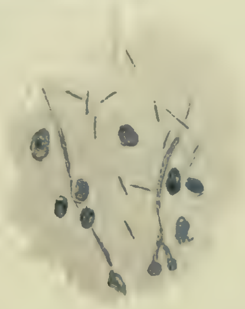

Fig. 1.

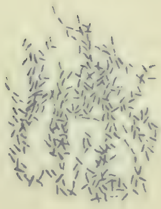

Fig. 2.

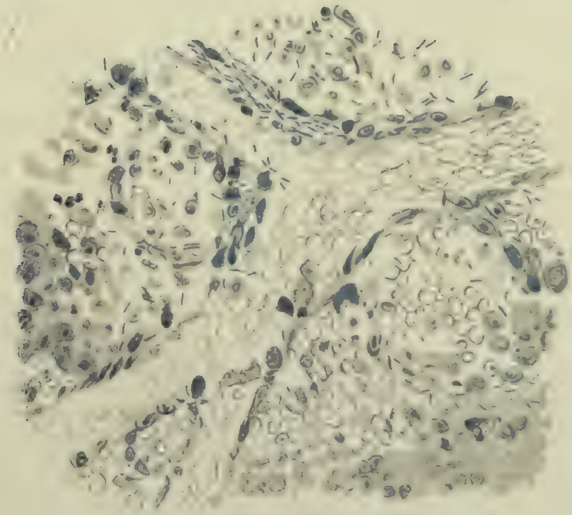

Fig 3.

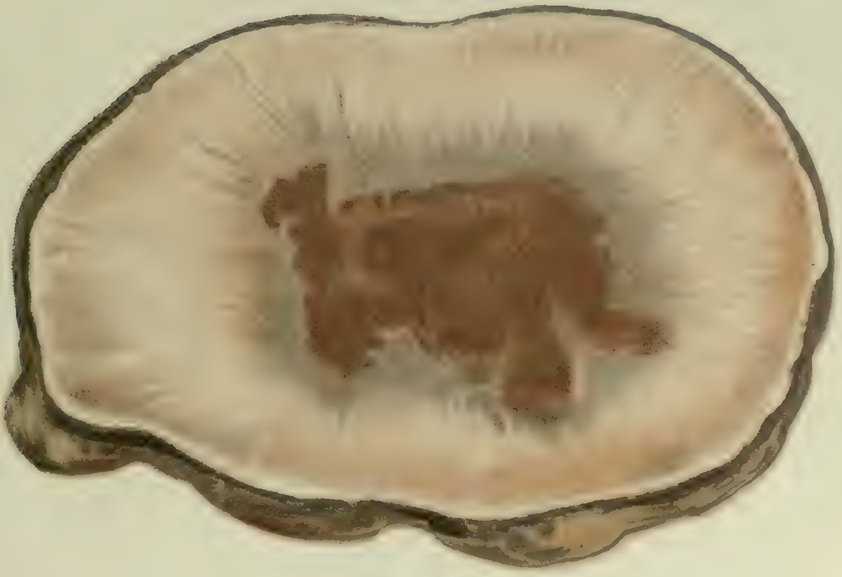

Fig. 4.

BACILLUS OF GLANDERS (LOEFFLER)

is viscid, drawing out into threads. In the absence of oxygen the characters of growth are the same as in its presence. The cultures acquire an alkaline reaction; they are sterilized by exposure for ten minutes to a temperature of 60° C. Does not grow upon *potato*.

Pathogenesis.—Pathogenic for chickens and turkeys. Not pathogenic for pigeons, guinea-pigs, or rabbits when injected subcutaneously or into the peritoneal cavity, but kills rabbits when injected into a vein. In the infected fowls the bacilli are found in small numbers in the blood, more numerous in the kidneys and liver, still more numerous in the spleen, and in enormous numbers in the intestinal mucus, where in acute cases it is found almost in a pure culture. Fowls do not contract the disease as a result of the ingestion of grains soiled with cultures of the bacillus, but become infected when fed with animal food to which a pure culture has been added.

XIV.

PATHOGENIC AËROBIC BACILLI NOT DESCRIBED IN PREVIOUS SECTIONS.

A CONSIDERABLE number of saprophytic bacilli are pathogenic for small animals when injected into the circulation, or subcutaneously, or into a serous cavity in considerable quantity—one to five cubic centimetres or more—but fail to produce any appreciable effect when introduced into the bodies of these animals in minute doses, and do not multiply in the blood to any considerable extent, although in fatal cases they may usually be recovered in cultures from the blood and tissues. These bacilli are pathogenic by reason of the toxic ptomaines produced by them, or because of local inflammatory processes which they induce, or for both of these reasons combined. Some of them may also, under certain circumstances, multiply in the blood and thus give rise to septicæmia as well as to toxæmia; this is the case, for example, with the "colon bacillus" of Escherich. When injected in considerable quantity into the circulation of a guinea-pig it causes the death of the animal within twenty-four hours, and the bacillus is found in the blood in great numbers; but minute amounts injected into a vein, or larger amounts injected subcutaneously, do not usually produce general infection. It is, therefore, not included among the "bacilli which produce septicæmia in susceptible animals." There is reason to believe, however, that under certain circumstances this bacillus may have sufficient pathogenic potency to produce a genuine septicæmia in guinea-pigs. Thus the original cultures of Brieger's bacillus, which appears to be a variety of the colon bacillus, are reported to have produced fatal septicæmia in guinea-pigs when injected subcutaneously in small amounts. A strict division into pathogenic bacilli which produce general blood infection—septicæmia—and those which produce a fatal result owing to the production of toxic chemical substances is not possible; for many pathogenic bacteria produce general infection when injected in comparatively large doses, and at the same time give rise to symptoms of toxæmia; or general infection may occur in animals of one species, and fatal toxæmia without septicæmia in those of another species. Many of the bacilli described in the present section are common saprophytes, which have been shown by laboratory experiments to be pathogenic for certain animals when introduced into their bodies in a certain amount, which differs greatly for different bacteria and for different species of animals. The experiments of Cheyne and others show how largely the pathogenic power of saprophytic bacteria depends upon the quantity of a culture which is injected, as well as upon the age of the culture and the seat of the inoculation—in the blood, the abdominal cavity, the subcutaneous tissues, or the muscles. And the bacteriologist named has also shown that pathogenic power depends, in some instances at least, upon the combined action of the toxic substances introduced in the first instance and of the living bacteria. Thus Cheyne found that one-tenth of a cubic centimetre of a bouillon culture of Proteus vulgaris injected into the dorsal muscles of a rabbit infallibly caused its death within forty-eight hours, but when the dose was reduced to one-fortieth cubic centimetre the animal recovered. But if to this amount (one-fortieth cubic centimetre) he added one cubic centimetre of a sterilized (by heat) culture of the same bacillus instead of diluting with distilled water, and injected the mixture into the dorsal muscles of a rabbit, death occurred in every experiment within forty-eight hours. The sterilized culture injected by itself produced no effect in this dose (one cubic centimetre), and Cheyne believes that the fatal result in these experiments was due to the fact that the toxic products present in the sterilized culture overcame the natural resisting powers of the tissues and enabled the bacillus to multiply over a larger area than would otherwise have been the case. As a result of this, toxic substances were produced in the body of the animal in sufficient quantity to cause general toxæmia and death; whereas the bacilli alone, in the dose mentioned, were not able to invade the tissues in the vicinity of the point of inoculation, and gave rise to a local abscess only. The same explanation is probably true for very many of the saprophytic bacteria which have been shown to possess pathogenic power; and it is probable that many of those which are now classed by bacteriologists as non-pathogenic would prove to be pathogenic in the same way if thoroughly tested upon various species of animals, although it might be necessary to use unusually large doses to accomplish the same result.

BACILLUS COLI COMMUNIS.

Synonyms.—Bacterium coli commune (Escherich); Colon bacillus of Escherich; Emmerich's bacillus (Bacillus Neapolitanus). Probably identical with Bacillus cavicida (Brieger's bacillus).

Obtained by Emmerich (1885) from the blood, various organs, and the alvine discharges of cholera patients at Naples; by Weisser (1886) from normal and abnormal human fæces, from the air, and from putrefying infusions; by Escherich (1886) from the fæces of healthy children; since shown to be commonly present in the alvine discharges of healthy men, and probably of many of the lower animals. Found by the writer in the blood and various organs of yellow-fever cadavers, in Havana (1888 and 1889).

Numerous varieties have been cultivated by different bacteriologists, which vary in pathogenic power and to some extent in their growth in various culture media; but the differences described are not sufficiently characteristic or constant to justify us in considering them as distinct species.

Morphology.—Differs considerably in its morphology as obtained from different sources and in various culture media. The typical form is that of short rods with rounded ends, from two to three μ in length and 0.4 to 0.6 μ broad; but under certain circumstances the length does not exceed the breadth—about 0.5 μ—and it might be mistaken for a micrococcus; again the prevailing form in a culture is a short oval; filaments of five μ or more in length are often observed in cultures, associated with short rods or oval cells. The bacilli are frequently united in pairs. The presence of spores has not been demonstrated. In unfavorable culture media the bacilli, in stained preparations, may present unstained places, which are supposed by Escherich to be due to degenerative changes in the protoplasm. Under certain circumstances some of the rods in a pure culture have been observed by Escherich to present spherical, unstained portions at one or both extremities, which closely resemble spores, but which he was not able to stain by the methods usually employed for staining spores, and which he is inclined to regard as "involution forms."

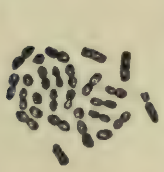

FIG. 144.—Bacillus coli communis. × 1 000. (Escherich.)

This bacillus *stains* readily with the aniline colors usually employed by bacteriologists, but quickly parts with its color when treated with iodine solution—Gram's method—or with diluted alcohol.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying* bacillus. Sometimes exhibits independent movements, which are not very active. One rod of a pair, in a hanging-drop culture, may advance slowly with a to-and-fro movement, while the other follows as if attached to it by an invisible band (Escherich). The writer's personal observations lead him to believe that, as a rule, this bacillus does not exhibit independent movements. Does not form spores. Grows in various culture media at the room temperature—more rapidly in the incubating oven. Grows in a decidedly acid medium.

In *gelatin plates* colonies are developed in from twenty-four to forty-eight hours, which vary considerably in their appearance according to their age, and in different cultures in the same medium. The deep colonies are usually spherical and at first are transparent, homogeneous, and of a pale-straw or amber color by transmitted light; later they frequently have a dark-brown, opaque central portion surrounded by a more transparent peripheral zone; or they may be coarsely granular and opaque; sometimes they have a long-oval or "whetstone" form. The superficial colonies differ still more in appearance; very young colonies by transmitted light often resemble little drops of water or fragments of broken glass; when they have sufficient space for their development they quickly increase in size, and may attain a diameter of three to four centimetres; the central portion is thickest, and is often marked by a spherical nucleus of a dark-brown color when the colony has started below the surface of the gelatin; the margins are thin and transparent, the thickness gradually increasing towards the centre, as does also the color, which by transmitted light varies from light straw color or amber to a dark brown. The outlines of superficial colonies are more or less irregular, and the surface may be marked by ridges, fissures, or concentric rings, or may be granular. The writer has observed colonies resembling a rosette, or a daisy with expanded petals. Escherich speaks of colonies which present star-shaped figures surrounded by concentric rings.

In *gelatin stab cultures* the growth upon the surface is rather dry, and may be quite thin, extending over the entire surface of the gelatin, or it may be thicker with irregular, leaf-like outlines and with superficial incrustations or concentric annular markings. An abundant development occurs all along the line of puncture, which in the deeper portion of the gelatin is made up of more or less closely crowded colonies; these are white by reflected light, and of an amber or light-brown color by transmitted light; later they may become granular and opaque. Frequently a diffused cloudy appearance is observed near the surface of the gelatin, and under certain circumstances branching, moss-like tufts develop at intervals along the line of growth. One or more gas bubbles may often be seen in recent stick cultures in gelatin.

Upon nutrient agar and blood serum, in the incubating oven, an abundant, soft, white layer is quickly developed. Upon *potato* an abundant, soft, shining layer of a brownish-yellow color is developed. The growth upon potato differs considerably, according to the age of the potato. According to Escherich, upon old potatoes there may be no growth, or it may be scanty and of a white color. In *milk*, at 37° C., an acid reaction and coagulation of the casein are produced at the end of eight or ten days. In the absence of oxygen this bacillus is able to grow in solutions containing grape sugar (Escherich). In bouillon it grows rapidly, producing a milky opacity of the culture liquid. The thermal death-point of Emmerich's bacillus, and of the colon bacillus from fæces, was found by Weisser to be 60° C., the time of exposure being ten minutes. The writer has obtained corresponding results. Weisser found that when the bacilli from a bouillon culture were dried upon thin glass covers they failed to grow after twenty-four hours. These results give confirmation to the view that the bacillus under consideration does not form spores. This view receives further support from the experiments of Walliczek (1894), who found that when dried upon pieces of sterile filter paper the bacillus failed to grow at the end of eighteen hours.

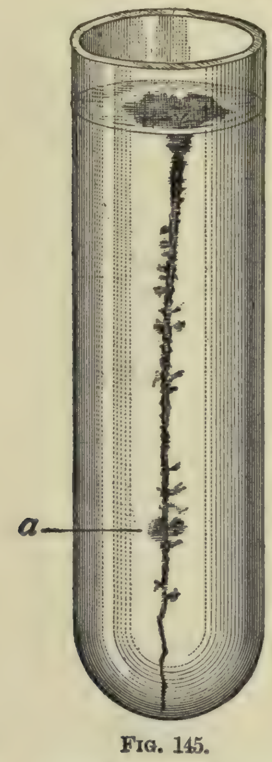

FIG. 145.

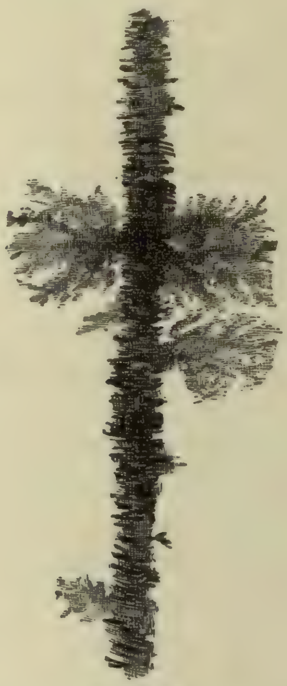

FIG. 146.

FIG. 145.—Bacillus coli communis in nutrient gelatin containing twenty per cent of gelatin, end of two weeks, showing moss-like tufts along the line of growth. (Sternberg.)

FIG. 146.—A portion of the growth shown in Fig 147, at *a*, magnified about six diameters. From a photograph. (Sternberg.)

Pathogenesis.—Comparatively small amounts of a pure culture of the colon bacillus injected into the circulation of a guinea-pig usually cause the death of the animal in from one to three days, and the bacillus is found in considerable numbers in its blood. But when injected subcutaneously or into the peritoneal cavity of rabbits or guinea-pigs, a fatal termination depends largely on the quantity injected; and although the bacillus may be obtained in cultures from the blood and the parenchyma of the various organs, it is not present in large numbers, and death appears to be due to toxæmia rather than to septicæmia. Mice are not susceptible to infection by subcutaneous injections. Small quantities injected beneath the skin of guinea-pigs usually produce a local abscess only; larger amounts—two to five cubic centimetres—frequently produce a fatal result, with symptoms and pathological appearances corresponding with those resulting from intravenous injection. These are fever, developed soon after the injection, diarrhœa, and symptoms of collapse appearing shortly before death. At the autopsy the liver and spleen appear normal, or nearly so; the kidneys are congested and may present scattered punctiform ecchymoses (Weisser). According to Escherich, the spleen is often somewhat enlarged. The small intestine is hyperæmic, especially in its upper portion, and the peritoneal layer presents a rosy color; the mucous membrane gives evidence of more or less intense catarrhal inflammation, and contains mucus, often slightly mixed with blood. In rabbits death occurs at a somewhat later date, and diarrhœa is a common symptom. In dogs the subcutaneous injection of a considerable quantity of a pure culture may give rise to an extensive local abscess.

In human pathology the colon bacillus plays an important *rôle.* It is concerned in the etiology of a considerable proportion of the cases of cystitis and of pyelonephritis, and peritonitis resulting from perforation. It appears to be the cause of certain affections of the anal region (Hartmann and Lieffring). It has been obtained in pure culture from abscesses in various parts of the body, from the valves of the heart in endocarditis, from the pleural cavity in empyema, etc. It has also been found in the blood, as a result of general infection following cystitis and pyelonephritis (Sittmann and Barnow).

Varieties.—Booker, in his extended studies relating to the bacteria present in the fæces of infants suffering from summer diarrhœa, has isolated seven varieties "which closely resemble Bacterium coli commune in morphology and growth in agar, neutral gelatin, and potato, but by means of other tests a distinction can be made between them."

Some of the pathogenic bacteria heretofore described are also closely allied to the "colon bacillus" and by some bacteriologists are supposed to belong to the same group—*i.e.*, to be varieties of the same species rather than independent species with fixed characters. Whatever may be the remote relationship, the typhoid group, the hog-cholera group, the Bacillus typhi murium of Löffler, the bacillus of Laser, the Bacillus enteritidis of Gärtner, and other similar bacilli appear to be differentiated from one another by characters which justify their description under separate names. Still it is difficult to fix upon any one of these characters to which specific value can be attached; and, in view of the many varieties found in nature or produced artificially in laboratory experiments, we are not justified in asserting that our classification of these low organisms has any substantial scientific foundation. The difficulties attending an attempt to establish specific characters are well illustrated by the extensive literature relating to the differentiation of bacilli belonging to the typhoid group from those belonging to the colon group. The main points upon which the distinction must depend have been referred to in the section devoted to the typhoid bacillus.

Fremlin (1893) has made a comparative study of the colon bacillus from various sources. He finds the common characters of gas production in media containing sugar and coagulation of milk. Cultivated from different animals the morphology is the same, but there are differences as regards motility. The most active movements are said to be exhibited in the bacillus from man, while the variety obtained from the intestines of rabbits showed scarcely any movements. The different varieties displayed considerable differences in their growth upon potato.

Dreyfuss (1894) finds decided differences in the pathogenic virulence of the colon bacillus from healthy individuals and from those suffering from various intestinal disorders. A culture from the discharges of a fatal case of cholera nostras proved to be exceptionally virulent—tested by intraperitoneal injections in guinea-pigs. Gilbert (1895), as a result of his extended researches, concludes that there are five principal types among the bacilli most nearly related to the colon bacillus: 1st. Bacilli which differ from the colon bacillus by their being non-motile. This type includes two varieties: one gives thick yellowish colonies upon gelatin plates and numerous gas bubbles on potato—this is the *bacille lactique* of Pasteur and the Bacillus lactis aërogenes of Escherich; the other gives thin, bluish-white colonies and includes the *bacille de l'éndocardite* of Gilbert and Lion. 2d. Bacilli which differ from the colon bacillus by the fact that cultures do not give the indol reaction. 3d. Bacilli which do not cause the fermentation of lactose. 4th. Bacilli which are not motile and do not ferment lactose. 5th. Bacilli which are not motile, do not give the indol reaction, and do not ferment lactose.

Theobald Smith (1895) gives the following account of his method of detecting bacilli of the "colon group" in water :

"The method followed by the writer in the general bacteriological examination of water consists, first, in the preparation of gelatin plates for the usual enumeration; and, second, in the addition to every one of ten fermentation tubes, containing a one-per-cent dextrose bouillon, a certain quantity of water. This is added most easily by first diluting the water, so that one or two cubic centimetres are equivalent to the quantity which it is desired to add to each tube. Pipettes graduated by drops are convenient, but not so accurate. In case of ground water it is well to prepare in addition a flask containing fifty to one hundred cubic centimetres of the water, and an equal, or greater, quantity of bouillon, to which sugar is *not* added. Plates may be prepared from this flask after sixteen to twenty-four hours. When gas begins to appear in the fermentation tubes, the amount accumulated at the end of each twenty-four hours should be marked with a glass pencil on the tube. From these tubes, which contain fifty to sixty per cent of gas on the third day, and are very strongly acid, plates may be prepared to confirm the indications of Bacillus coli. This, however, is not essential, for the writer has found as yet no species having these fermentative characters which is not one of the following: Bacillus coli, Bacillus lactis aërogenes, Bacillus enteriditis, Bacillus typhi murium, Bacillus choleræ suis. The three last-mentioned species are probably as rare in water as Bacillus typhosus itself.

"My own experience coincides with that of Matthews when he states that ninety-two per cent of all bacteria in ground water are suppressed in the thermostat. While the addition of 0.5 cubic centimetre, or even more, of such water may fail to produce cloudiness in any of the series of fermentation tubes, the same quantity, or less, of surface water never fails to infect the tubes."

Bacillus Coli Communis in Peritonitis.—The researches of A. Fränkel show that Bacillus coli communis may be obtained in pure cultures from the exudate into the peritoneal cavity in a considerable proportion of the cases of peritonitis, and there is good reason for believing that in these cases it was the cause of the inflammatory process. Thirty-one cases were examined by Fränkel, with the following result: Pure cultures of Bacillus coli communis were obtained in nine cases; of Streptococcus (pyogenes ?) in seven; of Bacillus lactis aërogenes in two; of "diplococcus pneumoniæ" in one; of Staphylococcus pyogenes aureus in one. Of the remaining eleven cases, seven gave mixed cultures, and in three of these Bacillus coli communis was the most abundant species. The author referred to has also shown that pure cultures of Bacillus coli communis injected into the cavity of the abdomen of rabbits cause a typical peritonitis. The present writer has frequently obtained the same result in experiments made with this bacillus. It would appear, therefore, that the peritonitis which so constantly results from wounds of the intestine is probably due, to a considerable extent, to the introduction of this microörganism from the lumen of the intestine, where it is constantly found, into the peritoneal cavity, where the conditions are favorable for its rapid development.

BACILLUS LACTIS AËROGENES.

Obtained by Escherich (1886) from the contents of the small intestine of children and animals fed upon milk; in smaller numbers from the fæces of milk-fed children, and in one instance from uncooked cow's milk.

Morphology.—Short rods with rounded ends, from 1 to 2 μ in length and from 0.1 to 0.5 μ broad; short oval and spherical forms are also frequently observed, and, under certain circumstances, longer rods —3 μ—may be developed: usually united in pairs, and occasionally in chains containing several elements. In some of the larger cells Escherich has observed unstained spaces, but was not able to obtain any evidence that these represent spores.

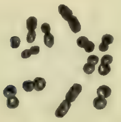

FIG. 147.—Bacillus lactis aërogenes. × 1,000. Escherich.)

This bacillus *stains* readily with the ordinary aniline colors, but does not retain its color when treated by Gram's method.

Biological Characters.—An *aërobic* (facultative anaërobic), *non-liquefying*, *non motile* bacillus. Does not form spores. Grows in various culture media at the room temperature—more rapidly in the incubating oven. Upon *gelatin plates*, at the end of twenty-four hours, small white colonies are developed. Upon the surface these form hemispherical, soft, shining masses which, examined under the microscope, are found to be homogeneous and opaque, with a whitish lustre by reflected light. The deep colonies are spherical and opaque and attain a considerable size. In gelatin stab cultures the growth resembles that of Friedländer's bacillus—*i.e.*. an abundant growth along the line of puncture and a rounded mass upon the surface, forming a "nail-shaped" growth. In old cultures the upper portion of the gelatin is sometimes clouded, and numerous gas bubbles may form in the gelatin. Upon the surface of nutrient agar an abundant, soft, white layer is developed. Upon old *potatoes*, in the incubating oven, at the end of twenty-four hours a yellowish-white layer, several millimetres thick, is developed, which is of paste-like consistence and contains about the periphery a considerable number of small gas bubbles; this layer increases in dimensions, has an irregular outline, and larger and more numerous gas bubbles are developed about the periphery, some the size of a pea; later the whole surface of the potato is covered with a creamy, semi-fluid mass filled with gas bubbles. On young potatoes the development is different; a rather luxuriant, thick, white or pale-yellow layer is formed, which is tolerably dry and has irregular margins; the surface is smooth and shining, and a few minute gas bubbles only are formed after several days.

Pathogenesis.—Injections of a considerable quantity of a pure culture into the circulation of rabbits and of guinea-pigs give rise to a fatal result within forty-eight hours.

In his first publication relating to "the bacteria found in the dejecta of infants afflicted with summer diarrhœa," Booker has described a bacillus which he designates by the letter B, which closely resembles Bacillus lactis aërogenes and is probably identical with it. He says:

"*Summary of Bacillus B.*—Found nearly constantly in cholera infantum and catarrhal enteritis, and generally the predominating form. It appeared in larger quantities in the more serious cases. It was not found in the dysenteric or healthy fæces. It resembles the description of the Bacillus lactis aërogenes, but the resemblance does not appear sufficient to constitute an identity, and, in the absence of a culture of the latter for comparison, it is considered a distinct variety for the following reasons: Bacillus B is uniformly larger, its ends are not so sharply rounded, and in all culture media long, thick filaments are seen, and many of the bacilli have the protoplasm gathered in the centre, leaving the poles clear. There is some difference in their colony growth on gelatin, and in gelatin stab cultures bacillus B does not show the nail-form growth with marked end swelling in the depth. In potato cultures the Bacillus lactis aërogenes shows a difference between old and new potatoes, while bacillus B does not show any difference.

"Bacillus B possesses decided pathogenic properties, which was shown both by hypodermic injections and feeding with milk cultures."

BACILLUS ACIDIFORMANS.

Obtained by the writer (1888) from a fragment of yellow-fever liver preserved for forty-eight hours in an antiseptic wrapping; since obtained from liver preserved in the same way from two comparative autopsies—*i.e.*, not cases of yellow fever.

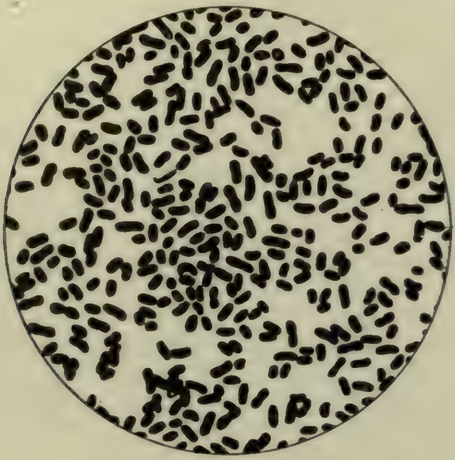

FIG. 148.

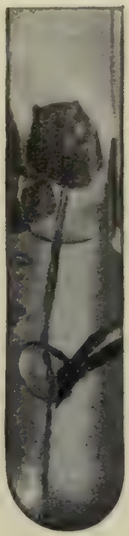

FIG. 149.

FIG. 148.—Bacillus acidiformans, from a potato culture. × 1,000. From a photomicrograph. (Sternberg.)

FIG. 149.—Culture of Bacillus acidiformans in nutrient gelatin, end of four days at 22° C. From a photograph. (Sternberg.)

Morphology.—A short bacillus with rounded corners, sometimes short oval in form; from 1½ to 3 μ in length and about 1.2 μ in breadth; may grow out into filaments of 5 to 10 μ, or more, in length; in some cultures the short oval form predominates.

Stains readily with the aniline colors usually employed, and by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying, non-motile* bacillus. Does not form spores. Grows rapidly at the room temperature in the usual culture media. Grows in decidedly acid media; in culture media containing glycerin or glucose it produces an abundant evolution of carbon dioxide, and a volatile acid is formed.

It does not liquefy gelatin, and in stab cultures grows abundantly both on the surface and along the line of puncture. At the end of twenty-four hours, at 22° C., a rounded white mass is formed upon the surface, resembling the growth of Friedländer's bacillus; at the bottom of the line of puncture the separate colonies are spherical, opaque, and pearl-like by reflected light. Gas bubbles are formed in the gelatin. At the end of a week the surface is covered with a thick, white, semi-fluid mass.

In gelatin roll tubes the superficial colonies are translucent or opaque, and circular or somewhat irregular in outline; by reflected light they are slightly iridescent; the deep colonies are spherical, opaque, and homogeneous.

The growth upon the surface of *nutrient agar* is abundant and rapid, of a shining milk-white color, and cream-like in consistence. An abundant development forms along the line of puncture and the culture medium is split up by gas bubbles. In glycerin-agar the evolution of gas is very abundant and the culture medium acquires an intensely acid reaction.

On *potato* the growth is abundant and rapid at a temperature of 20° to 30° C., forming a thick, semi-fluid mass of a milk-white color.

I have not obtained any evidence that this bacillus forms spores; the cultures are sterilized by ten minutes' exposure to a temperature of 160° F.

When cultivated in bouillon to which five per cent of glycerin has been added the culture medium acquires a milky opacity, and there is a copious precipitate, of a viscid consistence, consisting of bacilli; during the period of active development the surface is covered with gas bubbles, as in a saccharine liquid undergoing alcoholic fermentation, and the liquid has a decidedly acid reaction.

Pathogenesis.—Pathogenic for rabbits and for guinea pigs when injected into the cavity of the abdomen—one to two cubic centimetres of a culture in bouillon. The animal usually dies in less than twenty-four hours. The bacilli are found in the blood in rather small numbers, and are frequently seen in the interior of the leucocytes. The spleen is enlarged, the liver normal, the intestine usually hyperæmic.

BACILLUS CUNICULICIDA HAVANIENSIS.

Obtained by the writer (1889) from the contents of the intestine of yellow-fever cadavers, and also from fragments of yellow-fever liver preserved for forty-eight hours in an antiseptic wrapping—my bacillus *x*, Havana, 1889.

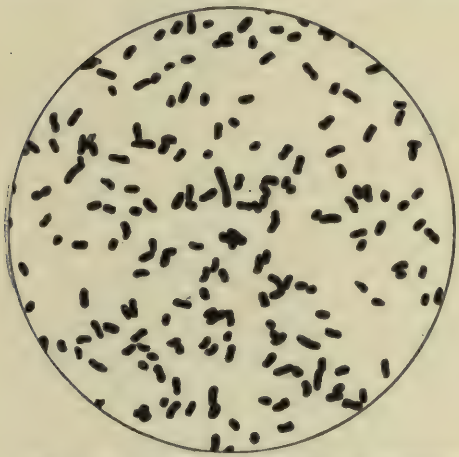

FIG. 150.—Bacillus cuniculicida Havaniensis, from a single colony in nutrient gelatin. × 1,000. From a photomicrograph. (Sternberg.)

Morphology.—This bacillus resembles the colon bacillus in form, but is somewhat larger, from 2 to 4 μ in length and from 0.8 to 1 μ in diameter; sometimes associated in pairs; may grow out into short filaments—not common. The ends of the rods are rounded, and under certain circumstances vacuoles are seen at the extremities, especially in potato cultures.

Stains quickly with the aniline colors usually employed, and also by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying* bacillus. Under certain circumstances may exhibit active movements, but is usually motionless.

A very curious thing with reference to this bacillus is that it presented active movements in my cultures made directly from yellow-fever cadavers, but that these movements were not constant, and that since my return to Baltimore I have not, as a rule, observed active movements in cultures from the same stock, which, however, preserved their pathogenic power and other characters. In Havana these movements were usually not observed in all the bacilli in a field under observation, but one and another would start from a quiescent condition on an active and erratic course; sometimes spinning actively upon its axis, and again shooting across the field as if propelled by a flagellum.

My notes indicate that cultures passed through the guinea-pig are more apt to be motile.

In gelatin stab cultures the growth of bacillus x resembles that of the colon bacillus, but the colonies at the bottom of the line of puncture are more opaque and not of a clear amber color like that of colonies of the colon bacillus. Upon the surface the growth is thicker than that of the colon bacillus, and forms a milk-white, soft mass.

The *colonies* in gelatin Esmarch roll tubes vary considerably at different times. Deep colonies are usually spherical, homogeneous, light brown in color, and more opaque than the similar colonies of the colon bacillus. At the end of a few days the deep colonies become quite opaque, and may be lobate, like a mulberry, or coarsely granular; sometimes the deep colonies have an opaque central portion surrounded by a transparent marginal zone.

In old gelatin roll tubes these deep colonies form opaque white hemispheres projecting from the surface of the dried culture medium, and little tufts of acicular crystals are sometimes observed to project from the side of such old colonies.

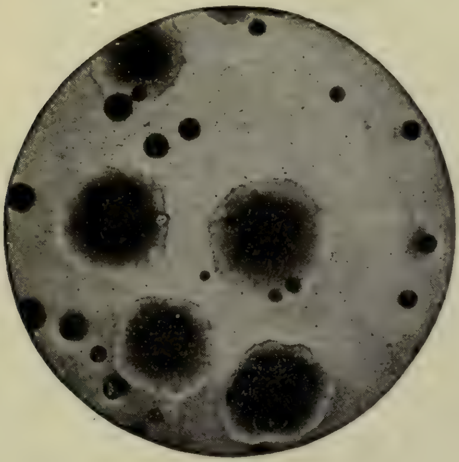

FIG. 151.

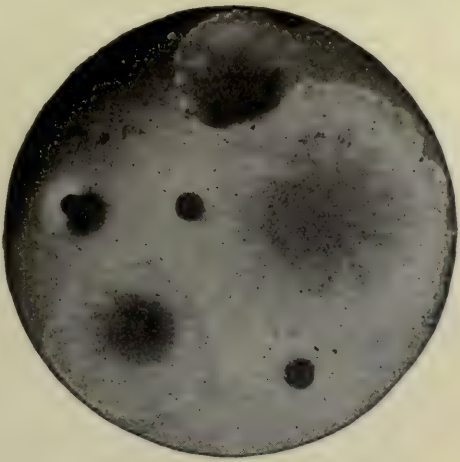

FIG. 152.

Fig. 151.—Bacillus cuniculicida Havaniensis; colonies in gelatin roll tube, third day at 20° C. × 6. From a photograph. (Sternberg.)

FIG. 152.—Bacillus cuniculicida Havaniensis; colonies in gelatin roll tube, end of forty-eight hours. × 10. From a photograph. (Sternberg.)

The superficial colonies are circular or irregular in outline, with transparent margins and an opaque central portion, sometimes corrugated. They are finely granular and iridescent by reflected light, and of a milk-white color; by transmitted light they have a brownish color. Young colonies closely resemble those of the colon bacillus. This bacillus grows well at a temperature of 20° C. (68° F.), but more rapidly and luxuriantly at a higher temperature—30° to 35° C.

It grows well in agar cultures, and especially in *glycerin-agar*, in which it produces some gas and an acid reaction. The growth on the surface of glycerin-agar cultures is white, cream-like in consistence, and quite abundant.

It grows well in an agar or gelatin medium made acid by the addition of 0.2 per cent (1:500) of hydrochloric acid.

In cocoanut water it multiplies rapidly, producing a milky opacity of the previously transparent fluid, an acid reaction, and an evolution of carbon dioxide.

On *potato* it produces a thick layer, which may cover the entire surface in three or four days, and which has a dirty-white, cream-white, or pinkishwhite color and cream-like consistence. The growth upon potato varies at different times, evidently owing to differences in the potato.

When stained preparations are examined with the full light of the Abbe condenser the ends of some of the rods appear to be cut away, leaving a concave extremity; but by using a small diaphragm to obtain definition it will be seen that the cell wall extends beyond the stained portion of the rod and includes what appears to be a vacuole. There is no reason to believe that this appearance is due to the presence of an end spore, for the supposed vacuole is not refractive, as a spore would be, and my experiments on the thermal death-point of this bacillus indicate that it does not form spores. Cultures are sterilized by exposure for ten minutes to a temperature of 160° F. (71.2° C.).

Pathogenesis.—Very pathogenic for rabbits when injected into the cavity of the abdomen. Injections of a small quantity of a pure culture into the ear vein or subcutaneously generally give a negative result. Injections of from one to five cubic centimetres of a culture in bouillon, blood serum, or agua coco, into the cavity of the abdomen, frequently prove fatal to rabbits in a few hours—two to six.

The negative results obtained in injecting cultures beneath the skin or into the ear vein of rabbits show that this bacillus does not induce a fatal septicæmia in these animals, and the fatal result when injections are made into the peritoneal cavity does not appear to be due to an invasion of the blood, but rather to the local effect upon the peritoneum, together with the toxic action of the chemical products resulting from its growth.

It is true that I have always been able to recover the bacillus from the liver, or from blood obtained from one of the cavities of the heart, even in animals which succumb within a few hours to an injection made into the cavity of the abdomen. But the direct examination of the blood shows that the bacilli are present in very small numbers, and leads me to believe that the bacillus does not multiply, to any considerable extent at least, in the circulating fluid.

The spleen is not enlarged, as is the case in anthrax, rabbit septicæmia, and other diseases in which the pathogenic microörganism multiplies abundantly in the blood.

On the other hand, there is evidence of local inflammation in the peritoneal cavity. When death occurs within a few hours the peritoneum is more or less hyperæmic and there is a considerable quantity of straw-colored fluid in the cavity of the abdomen. When the animal lives for twenty hours or more there is a decided peritonitis with a fibrinous exudation upon the surface of the liver and intestine. Usually the liver, in animals which die within twenty-four hours, is full of blood, rather soft, and dark in color. In a single instance I found the liver to be of a light color and loaded with fat.

The rapidly fatal effect in those cases in which I have injected two or more cubic centimetres of a culture into the cavity of the abdomen has led me to suppose that death results from the toxic effects of a ptomaine contained in the culture at the time of injection. The symptoms also give support to this supposition. The animal quickly becomes feeble and indisposed to move, and some time before death lies helpless upon its side, breathing regularly, but is too feeble to get up on its feet when disturbed. Death sometimes occurs in convulsions, but more frequently without—apparently from heart failure.

Pathogenic also for guinea-pigs when injected into the cavity of the abdomen, but death does not occur in so short a time—eighteen to twenty hours. The comparative researches of Reed and Carroll indicate that this is a pathogenic variety of the colon bacillus.

BACILLUS LEPORIS LETHALIS.

Obtained by Dr. Paul Gibier (1888) from the contents of the intestine of yellow-fever patients; also by the writer from the same source (1888, 1889) in exceptional cases and in comparatively small numbers. Named and described by present writer.

Morphology.—Bacilli with rounded ends, from 1 to 3 μ in length and about 0.5 μ in breadth. The length may vary in the same culture from a short oval to rods which are two or three times as long as broad, or it may grow out into flexible filaments of considerable length. In recent cultures the bacilli are frequently united in pairs.

Stains readily with the aniline colors usually employed. In cultures which are several days old, or in recent cultures when the stained preparation is washed in alcohol, the ends of the rods are commonly more deeply stained than the central portion—"end staining"; and in old cultures some of the bacilli are very faintly stained.

Biological Characters.—An *aërobic*, *liquefying*, *actively motile* bacillus. Does not form spores.

In *gelatin stab cultures*, at the end of twenty-four hours at a temperature of 20° to 22° C., there is an abundant development along the line of puncture and commencing liquefaction at the surface. Later the liquefaction is funnel-shaped, and there is an opaque white central core along the line of puncture, with liquefied gelatin around it. Liquefaction progresses most rapidly at the surface, and in the course of three or four days the upper portion of the gelatin for a distance of half an inch or more is completely liquefied, and an opaque white mass, composed of bacilli, rests upon the surface of the unliquefied portion.

In gelatin roll tubes the young colonies upon the surface are transparent and resemble somewhat small fragments of broken glass; later liquefaction occurs rapidly. Deep colonies in gelatin roll tubes, or at the bottom of stick cultures, are spherical, translucent, and of a pale straw color.

Upon the surface of nutrient agar it grows rapidly, forming a rather thin, translucent, shining, white layer, which covers the entire surface at the end of two or three days at a temperature of 20° C.

Upon *potato* the growth is rapid and thin, covering the entire surface, and is of a pale-yellow color.

This bacillus grows at a comparatively low temperature, and its vitality is not destroyed by exposure for an hour and a half in a freezing mixture at 15° C. below zero (5° F.).

Decided growth occurred in a stick culture in gelatin exposed in Baltimore during the month of January in an attic room. During the twenty-two days of exposure the highest temperature, taken at 9 A.M. each day, was 11° C., and the lowest 2° C. At a temperature of 16° to 20° C. development in a favorable culture medium is rapid.

There is no evidence that this bacillus forms spores; cultures are sterilized by exposure to a temperature of 60° C. for ten minutes.

Coagulated blood serum is liquefied by this bacillus. It retains its vitality for a long time in old cultures, having grown freely when replanted at the end of a year from a hermetically sealed tube containing a pure culture in blood serum.

Pathogenesis.—This bacillus is very pathogenic for rabbits when injected into the cavity of the abdomen in quantities of one cubic centimetre or more; it is less pathogenic for guinea-pigs, and is not pathogenic for white rats when injected subcutaneously. Gelatin cultures seem to possess more intense pathogenic power than bouillon cultures, and cultures from the blood of an animal recently dead as the result of an inoculation are more potent than those from my original stock which had not been passed through a susceptible animal.

The mode of death in rabbits is quite characteristic. A couple of hours after receiving in the cavity of the abdomen two or three cubic centimetres of a liquefied gelatin culture the animal becomes quiet and indisposed to eat or move about. Soon after it becomes somnolent, the head drooping forward and after a time resting between the front legs, with the nose on the floor of its cage. It can be roused from this condition, and raises its head in an indifferent and stupid way when pushed or shaken, but soon drops off again into a profound sleep. Frequently the animals die in a sitting position, with their nose resting upon the floor of the cage between the front legs. I have not seen this lethargic condition produced by inoculations with any other microörganism. Convulsions sometimes occur at the moment of death.

The time of death depends upon the potency of the culture and its quantity as compared with the size of the animal. From three to four cubic centimetres of a liquefied gelatin culture usually kill a rabbit in from three to seven hours.

The rapidity with which death occurs when a considerable quantity of a liquefied gelatin culture is injected into the cavity of the abdomen, and the somnolence which precedes death, give rise to the supposition that the lethal effect is due to the presence of a toxic chemical substance rather than to a multiplication of the bacillus in the body of the animal. And this view is supported by the fact that animals frequently recover when the dose administered is comparatively small and especially when it is injected subcutaneously.

In all cases in which death occurs, even when but a few hours have elapsed since the inoculation was made, I have recovered the bacillus in cultures made from blood obtained from the heart or the interior of the liver, and, as stated, these cultures appear to have a greater virulence than those not passed through the rabbit.

In sections of the liver and kidney stained with Löffler's solution of methylene blue the bacilli are seen, and are often in rather long-jointed filaments.

BACILLUS PYOCYANEUS.

Synonyms.—Bacillus of green pus ; Microbe du pus bleu; Bacillen des grünblauen Eiters ; Bacterium aëruginosum.

Obtained by Gessard (1882) from pus having a green or blue color, and since carefully studied by Gessard, Charrin, and others.

This bacillus appears to be a widely distributed saprophyte, which is found occasionally in the purulent discharges from open wounds, and sometimes in perspiration and serous wound secretions (Gessard). The writer obtained it, in one instance, in cultures from the liver of a yellow-fever cadaver (Havana, 1888).

FIG. 153. — Bacillus pyocyaneus. × 700. (Flügge.)

Morphology.—A slender bacillus with rounded ends, somewhat thicker than the Bacillus murisepticus and of about the same length (Flügge); frequently united in pairs, or chains of four to six elements; occasionally grows out into filaments.

Biological Characters.—An *aërobic, liquefying, motile* bacillus. Grows readily in various culture media at the room temperature—more rapidly in the incubating oven. Is a facultative anaërobic (Fränkel). Does not form spores. The thermal death-point, as determined by the writer, is 56° C., the time of exposure being ten minutes. In *gelatin plate cultures* colonies are quickly developed, which give to the medium a fluorescent green color; at the end of two or three days liquefaction commences around each colony, and usually the gelatin is completely liquefied by the fifth day. Before liquefaction commences the deep colonies, under a low power, appear as spherical, granular masses, with a serrated margin, and have a yellowish-green color; the superficial colonies are quite thin and finely granular; at the centre, where they are thickest, they have a greenish color, which fades out towards the periphery.

In *stab cultures* in nutrient gelatin development is most abundant near the surface, and causes at first liquefaction in the form of a shallow funnel; later the liquefied gelatin is separated from that which is not liquefied by a horizontal plane, and a viscid, yellowish-white mass, composed of bacilli, accumulates upon this surface, which gradually has a lower level as liquefaction progresses; the transparent, liquefied gelatin above is covered with a delicate, yellowish-green film, and the entire medium has a fluorescent green color. Upon nutrient *agar* a rather abundant, moist, greenish-white layer is developed, and the medium acquires a bright green-color, which subsequently changes to olive green. Upon *potato* a viscid or rather dry, yellowish-green or brown layer is formed, and the potato beneath and immediately around the growth has a green color when freely exposed to the air or to the vapors of ammonia. In *milk* the casein is first precipitated and then gradually dissolved, while at the same time ammonia is developed. The *green pigment* is formed only in the presence of oxygen; it is soluble in chloroform and may be obtained from a pure solution in long, blue needles; acids change the blue color to red, and reducing substances to yellow. According to Ledderhose, it is an aromatic compound resembling anthracene, and is not toxic. According to Gessard's latest researches (1890), two different pigments are produced by this bacillus, one of a fluorescent green and the other—pyocyanin—of a blue color. Cultivated in egg albumin the fluorescent green pigment, which changes to brown with time, is alone produced. In bouillon and in media containing peptone or gelatin both pigments are formed, and the pyocyanin may be obtained separately by dissolving it in chloroform. In an alkaline solution of peptone (two per cent) to which five per cent of glycerin has been added the blue pigment alone is formed.

Pathogenesis.—The experiments of Ledderhose, Bouchard, and others show that this bacillus is pathogenic for guinea-pigs and rabbits. Subcutaneous or intraperitoneal injections of recent cultures—

one cubic centimetre or more of a culture in bouillon—usually cause the death of the animal in from twelve to thirty-six hours. An extensive inflammatory œdema and purulent infiltration of the tissues result from subcutaneous inoculations, and a sero-fibrinous or purulent peritonitis is induced by the introduction of the bacillus into the peritoneal cavity. The bacillus is found in the serous or purulent fluid in the subcutaneous tissues or abdominal cavity, and also in the blood and various organs, from which it can be recovered in pure cultures, although not present in great numbers, as is the case in the various forms of septicæmia heretofore described. When smaller amounts are injected subcutaneously the animal usually recovers after the formation of a local abscess, and it is subsequently immune when inoculated with doses which would be fatal to an unprotected animal. Immunity may also be secured by the injection of a considerable quantity of a sterilized culture. Bouchard has also produced immunity in rabbits by injecting into them the filtered urine of other rabbits which had been inoculated with a virulent culture of the bacillus. It has been shown by Bouchard, and by Charrin and Guignard, that in rabbits which have been inoculated with a culture of the anthrax bacillus a fatal result may be prevented by soon after inoculating the same animals with a pure culture of the Bacillus pyocyaneus. The experiments of Woodhead and Wood indicate that the antidotal effect is due to chemical products of the growth of the bacillus, and not to an antagonism of the living bacterial cells. They were able to obtain similar results by the injection of sterilized cultures of Bacillus pyocyaneus, made soon after infection with the anthrax bacillus.

Schimmelbusch (1894) reports that in researches made by Mühsam this bacillus was found in the axilla, the anal region, or the inguinal fold in fifty per cent of the healthy individuals examined. Its presence in wounds greatly delays the process of repair and may give rise to a general depression of the vital powers from the absorption of its toxic products. Schimmelbush states that a physician injected 0.5 cubic centimetre of sterilized (by heat) culture into his forearm. That as a result of this injection, after a few hours he had a slight chill, followed by fever, which at the end of twelve hours reached 38.8° ; an erysipelatous-like swelling of the forearm occurred, and the glands in the axilla were swollen and painful. Recovery occurred without the formation of an abscess. Buchner has related a similar case.

Krannhals (1894) refers to seven cases in which a general pyocyaneus infection in man was found, and adds an eighth from his own experience. In this the Bacillus pyocyaneus was obtained, post mortem, from green pus in the pleural cavity, from serum in the pericardial sac, and from the spleen, in pure culture.

Martha, Gruber, Maggiora, Gradenigo, Kossel, and Rohrer have reported cases in which the Bacillus pyocyaneus has been obtained in pure cultures from pus obtained from the tympanic cavity in middle-ear disease. Kossel (1894) relates several cases in his own experience which led him to the conclusion that, in children, the Bacillus pyocyaneus, through general blood infection or indirectly through the absorption of its toxic products, may be the cause of death.

The following varieties of this bacillus have been described by bacteriologists:

BACILLUS PYOCYANEUS β (P. Ernst).

Found in pus from bandages colored green.

Morphology.—Slender bacilli from 2 to 4 μ long—occasionally 5 to 6 μ—and from 0.5 to 0.75 μ broad; sometimes united in pairs, or chains of three elements.

Biological Characters.—An *aërobic*, *liquefying*, *actively motile*, *chromogenic* bacillus. Produces a yellowish-green pigment; when old cultures are shaken up with chloroform and this is allowed to stand, three layers are formed—an upper, clouded, dirty-yellow layer; below this is a milky, pale-green layer; and at the bottom a transparent, azure-blue layer. Spore formation has not been demonstrated. Grows in the usual culture media at the room temperature—more rapidly at 35° C. Upon *gelatin plates* colonies are formed resembling those of the well-known Bacillus pyocyaneus, but liquefaction is more rapid. In *gelatin stick cultures* funnel-shaped liquefaction occurs at the upper part of the line of puncture by the third day, and progresses more rapidly than is the case with Bacillus pyocyaneus under the same circumstances; on the fifth day a bluish-green color is developed; by the twelfth day liquefaction has obliterated the entire line of growth and extends to the margins of the tube; the liquefied gelatin for a depth of about one centimetre has a dark emerald-green color, and a film consisting of bacilli is seen upon the surface. Upon the *surface of agar* a flat, greenish-white, dry layer is formed along the line of inoculation, and the agar around, at the end of a week, acquires a bluish-green color. Upon *potato*, at the end of three days, an abundant dry layer of a fawn-brown color has developed; this is surrounded by a pale-green coloration of the potato, and at points where the surface is fissured, an intense dark-green color is developed; the growth on potato has a more or less wrinkled appearance; when one of the fawn-colored colonies is touched with the platinum needle, the point touched, at the end of two to five minutes, acquires an intense dark leaf-green color, which reaches its maximum intensity in about ten minutes, and has faded out again at the end of half an hour. Ernst considers this "chameleon phenomenon" the most characteristic distinction between the bacillus under consideration and Bacillus pyocyaneus. In *milk* a green color is developed at the surface, the casein is precipitated and subsequently peptonized.

Bacillus pyocyaneus pericarditidis. Found by H. C. Ernst in fluid obtained by tapping the pericardial sac of a man aged forty-seven years. Fluid was drawn from the pericardial sac on four different occasions. The man subsequently "eloped." Ernst gives the following description of this bacillus:

ORIGIN.—Pericardial fluid, containing also bacilli of tuberculosis.

FORM AND ARRANGEMENT.—Small straight bacilli, with rounded ends, three or four times as long as broad, and on most media slightly larger than the Bacillus pyocyaneus of Gessard, occurring within the cells in the original fluid, and sometimes showing two or three end to end, but never observed in long chains.

MOTILITY.—Actively motile in hanging-drop culture. No cilia or flagella have been demonstrated.

GROWTH—*Gelatin: Plates.*—Colonies appear at the end of thirty-six to forty-eight hours as fine white points in the interior, and upon the surface of the medium; edges are sharply defined; soon there appears a circular zone of liquefaction, finally passing through the stratum of the medium with the colony at the bottom. Under a low power the centre of the colony may be of a brownish color. On the second day a greenish tinge may be seen about the individual colonies on the surface which spreads through the entire medium. The plates may always be distinguished from those of the Bacillus pyocyaneus of Gessard by the *bluish-green* when contrasted with the yellowish-green color of this latter.

Gelatin: Needle Cultures.—At the end of twenty-four hours a small, saucer-shaped depression of liquefaction at upper end of needle track, which gradually spreads and deepens until the liquefaction extends straight across the tube, and about half-way down the needle track. A bluish-green fluorescence appears about the liquefied portion at the very upper part of the gelatin, later changing into a yellowish green. The colony is deposited as a yellowish, heavy sediment at the bottom of the liquefied portion, the upper part of which is clear. A small, whitish growth occurs along the remainder of the needle track. Old cultures, in which a certain amount of evaporation has occurred, assume a very dark greenish-black color.

Agar-agar.—Along the needle track appears a *flat*, dry colony of a dirty grayish-white color spreading out upon each side of the needle track and growing at first upon the surface of the water of condensation, later depositing a white sediment at the bottom. From the first there may be detected, by reflected light, a metallic lustre on the surface of the colony in places, which metallic sheen later spreads over the whole colony and furnishes a marked differentiating point. In addition to this, within twenty-four to forty-eight hours at 37° C., there appears a green fluorescence throughout the whole of the medium, which increases slowly to a marked *bluish-green* color, and never assumes the nut-brown of the Bacillus pyocyaneus of Gessard upon the same medium. The colony is not especially viscid.

Potato.—There appears a reddish-brown colony along the needle track, elevated and moist, confined to the line of the needle. It presents no change of color upon touching with the needle, but certain specimens (as do some of the Bacillus pyocyaneus) develop later a heavy green color extending over the whole surface of the potato, which later changes almost to black.

Bouillon.—Twenty-four hours at 37° C. gives a growth, especially on the surface, which is a wrinkled scum; no cloudiness of the bouillon, and a very faint greenish fluorescence one centimetre below the surface. At this time it differs from the Bacillus pyocyaneus of Gessard, in that the latter shows cloudiness of the medium all through. Later the same cloudiness appears in bouillon cultures of this new bacillus, together with a whitish sediment deposited at the bottom of the tube, and then the cultures are indistinguishable from each other. The same changes, but slower, occur at room temperature.

Peptone.—One, 3.5, and six-per-cent solution. Twenty-four hours at 37° C. gives a faint bluish tinge at upper edge of medium with very faint cloudiness; later (in one or two weeks) there forms a marked scum upon the surface that is difficult to break up by shaking, and the whole medium assumes a grass-green color of more or less intensity, and not seen on other similar bacilli. The shape and size of the organism, under the microscope, differ very markedly in this medium from any other bacilli examined. The same changes are to be seen at room temperature, but more slowly.

Egg-Albumin: Plain.—Twenty-four hours at 37° C., yellowish-white, very profuse growth all along the needle track; yellowish-green spreading out from it almost to sides of tube, and in the condensation water as well. The growth has no especial distinguishing characteristics. Irregular liquefaction occurs, but the growth at no time differs in any marked way from other varieties of the Bacillus pyocyaneus.

Blood Serum.—Twenty-four hours at 37° C. shows flat, moist colony with bluish-green fluorescence in its neighborhood. Liquefaction begins early and goes on slowly until complete in from one to two weeks, with an increasing intensity of color which becomes markedly blue, and eventually almost black.

Milk.—Behaves as do the other bacteria.

BEHAVIOR TO TEMPERATURE.—Grows at 15°–25° C. slowly; much more freely at 35°–38° C., when it produces the color more quickly.

RAPIDITY OF GROWTH.—Moderate.

SPORE-PRODUCTION.—Not observed.

NEED OF AIR.—Does not grow under mica. Facultatively anaërobic, but does not produce color except with free access of oxygen.

GAS-PRODUCTION.—Produces faint foul odor.

BEHAVIOR TO GELATIN.—Liquefies gelatin slowly.

COLOR-PRODUCTION.—Produces a bluish-green color which in old cultures changes almost to a black. Upon the addition of acids (both vegetable and mineral) to cultures the color changes to red, and upon the addition of alkalies a bright grass-green appears. This reaction is best seen in bouillon and gelatin cultures, but occurs in other media as well, notably blood-serum.

BEHAVIOR TO ANILINE DYES.—Stains easily and well with any of the aniline dyes usually employed, and by Gram's method.

MICROSCOPIC APPEARANCE IN DIFFERENT MEDIA.—Under the microscope, its general appearance on various media is of a rod larger than the Bacillus pyocyaneus. In peptone cultures this difference is verv marked. In this case, the Bacillus pyocyaneus tested appeared as very short, oval, bacilli, almost like micrococci, while the new bacillus showed as a long, fine rod, from four to six times as long as broad—length about one-half the diameter of a red-blood corpuscle—and arranged sometimes two or three end to end. These same cultures transferred to gelatin became indistinguishable from each other in size.

PATHOGENESIS.—Injections of small quantities (0.5 centimetre) of a bouillon culture twenty-four hours old into the abdominal cavity of rabbits and guinea-pigs, killed fifty per cent in from twenty-four to thirty-six hours. Autopsy showed general congestion of abdominal viscera, slight effusion into the peritoneal cavity, and cover-glass preparations and cultures showed the bacilli in the effusion in the abdominal cavity, as well as in the blood from the heart and various organs.

BACILLUS OF FIOCCA.

Found by Fiocca in the saliva of cats and dogs.

Closely resembles the influenza bacillus of Pfeiffer and of Canon.

Morphology.—Resembles the bacillus of rabbit septicæmia, but is only half as large—from 0.2 to 0.33 μ in breadth. The length is but little greater than the breadth. Usually seen in pairs, closely resembling diplococci. When cultivated on potato it appears to be a micrococcus, but in the blood of infected animals and in bouillon cultures it is seen to be a short bacillus.

Stains with difficulty with the usual aniline colors, but is readily stained by Ehrlich's method or with Ziehl's solution.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying, non-motile* bacillus. Spore formation not observed. Grows best at 37° C. and does not develop at temperatures below 15° C. In agar plates, at 37° C., small, punctiform colonies are developed at the end of twenty-four hours; these do not increase in size later; under the microscope the deep colonies are seen to be spherical, granular, and dark yellow in color; the superficial colonies are more or less round, with irregular outlines, transparent, slightly granular, and often have a shining nucleus at the centre. Upon gelatin plates the colonies have a similar appearance, but are not visible in less than four or five days. In streak cultures upon the surface of agar small, punctiform colonies are seen along the track of the needle at the end of twenty-four hours, resembling fine dewdrops; the following day these colonies are a little larger and less transparent; they remain distinct, especially along the margins of the line of growth. Upon *potato* a very thin, transparent layer is developed, which does not change the appearance of the surface of the potato, but slightly increases its resistance to the platinum needle. In *bouillon* small flocculi, suspended in the clear liquid, are developed within twenty-four hours; these subsequently sink to the bottom.

Milk is not coagulated by this bacillus, and no gas is produced in media containing sugar.

Pathogenesis.—Pathogenic for rabbits, guinea-pigs, young rats, and mice, in which animals it produces general infection, and death—in rabbits—at the end of twenty-four hours. The bacillus is found in the blood in great numbers.

PROTEUS VULGARIS.

Obtained by Hauser (1885) from putrefying animal substances, and since shown to be one of the most common and widely distributed putrefactive bacteria. This and the other species of Proteus described by the same bacteriologist (Proteus mirabilis, Proteus Zenkeri) have no doubt frequently been encountered by previous observers, and are among the species formerly included under the name "Bacterium termo," which was applied to any minute motile bacilli found in putrefying infusions.

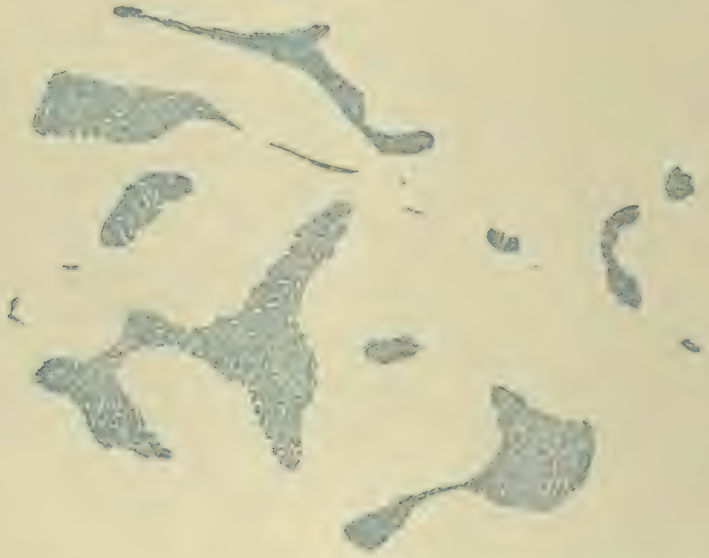

FIG. 154.—Proteus vulgaris; "swarming islands" from a gelatin culture. × 285. (Hauser.)

Morphology.—Bacilli with rounded ends, about 0.6 μ broad, and varying greatly in length, being sometimes short oval, and at others from 1.25 to 3.75 μ in length; also grow out into flexible filaments, which may be more or less wavy or spiral in form. The short rods are commonly seen in pairs; they have terminal flagella; involution forms are frequently seen, the most common being spherical bodies about 1.6 μ in diameter. In old cultures in bouillon, or in cultures made in meat infusion in the incubating oven, the short oval forms greatly predominate, but in recent cultures in nutrient gelatin filaments of considerable length are encountered in association with shorter rods.

Stains readily with fuchsin or gentian violet—not so well with the brown aniline colors; does not stain by Gram's method (Cheyne).

Biological Characters.—An *aërobic* and *facultative anaërobic, liquefying, motile* bacillus. Grows rapidly in the usual culture media at the room temperature.

The growth upon *gelatin plates* (five per cent of gelatin) at the room temperature is very characteristic; at the end of six or eight hours small depressions in the gelatin are observed, which contain liquefied gelatin and grayish-white masses of bacilli. Under a low power these depressions are seen to be surrounded by a marginal zone consisting of two or three layers, outside of which is a zone of a single layer, from which amœba-like processes extend upon the surface of the gelatin. These processes are constantly undergoing changes in their form and position, and may become separated from the mother colony, or remain temporarily attached to it by a narrow thread consisting of bacilli; after a time the entire surface of the gelatin is covered with wandering, amœba-like colonies; these rapidly cause liquefaction, which by the end of twenty-four to forty-eight hours has reached a depth of one millimetre or more over the entire surface. The deep colonies also are surrounded by processes projecting into the gelatin, which may be observed to suddenly advance and again to be retracted towards the central zoöglœa-like mass. Liquefaction around the colony rapidly progresses, and actively motile rods and spiral filaments may be seen about the periphery of this liquefied gelatin, while about it is a radiating crown of irregular processes, some of which may be screw-like or corkscrew-formed. In ten-per-cent gelatin the migration of surface colonies, above described, is not observed. In *gelatin stab cultures* liquefaction occurs along the entire line of puncture, and soon the contents of the tube are completely liquefied; near the surface of the liquefied gelatin the growing bacilli form a grayish-white cloudiness, and at the bottom of the tube an abundant flocculent deposit is formed. Upon the surface of *nutrient agar* a rapidly extending, moist, thin, grayish-white layer is formed. Upon *potato* this bacillus produces a dirty-white, moist layer. The cultures in media containing albumin or gelatin have a putrefactive odor and acquire a strongly alkaline reaction. A temperature of 20° to 24° C. is most favorable for the growth of this bacillus. It is a facultative anaërobic and grows in an atmosphere of hydrogen or of carbon dioxide, although not so rapidly as in the presence of oxygen. The movements are often extremely active and difficult to follow under the microscope ; again they may be quite deliberate, or the bacilli may remain motionless for a time and again dart off in active motion. The long terminal flagella may sometimes be discerned by means of a good objective and careful manipulation of the light.

Pathogenesis.—Pathogenic for rabbits and for guinea-pigs when injected into the circulation, into the cavity of the abdomen, or subcutaneously in considerable quantity. Cultures in nutrient gelatin are said by Cheyne to be more pathogenic (toxic) than those in bouillon. When injected into the muscles of rabbits a much smaller dose produces a fatal result than when injected subcutaneously. In Cheyne's experiments, made in London (1886), one-tenth cubic centimetre of a liquefied gelatin culture, injected into the dorsal muscles, was invariably fatal in from twenty-four to thirty-six hours; a dose of one-twentieth cubic centimetre, injected in the same way, usually caused death; while one-fortieth cubic centimetre gave rise to an extensive local abscess, and the animals died at the end of six or eight weeks. Doses of less than one-five-hundredth cubic centimetre produced no effect. Cheyne estimates that one cubic centimetre of a culture in nutrient gelatin contains 4,500,000,000 bacilli, and, consequently, that a smaller number than 9,000,000 produced no effect when injected into the muscular tissue of rabbits. Injections into the subcutaneous connective tissues of a dose twice as large as that which invariably proved fatal when injected into the muscles usually caused an extensive abscess, but did not kill the animal; and, after recovery from the effects of such an injection, the rabbit was found to be immune against a similar dose injected into the muscles. Foà and Bonome have succeeded in producing immunity against the effects of virulent cultures of this bacillus by inoculating rabbits with filtered cultures, and also by injecting beneath the skin of these animals a solution of neurin, which they believe to be the principal toxic product present in the cultures.

Proteus Vulgaris in Cholera Infantum.—The extended researches of Booker have led him to the conclusion that this bacillus plays an important part in the production of the morbid symptoms which characterize cholera infantum. Proteus vulgaris was found in the alvine discharges in a considerable proportion of the cases examined, but was not found in the fæces of healthy infants. "The prominent symptoms in the cases of cholera infantum in which the proteus bacteria were found were drowsiness, stupor, emaciation and great reduction in flesh, more or less collapse, frequent vomiting and purging, with watery and generally offensive stools."

The researches of Krogius, Schnitzler, Schmidt and Aschoff, and others, show that in cases of cystitis and of pyelonephritis this bacillus is frequently found in pure cultures, or associated with other bacteria. The authors last named state that in sixty cases of cystitis reported by various authors the colon bacillus was found in pure cultures, and in thirteen cases the proteus of Hauser. Next to Bacillus coli communis Proteus vulgaris appears to be the microörganism most frequently concerned in the etiology of pyelonephritis.

Levy (1895) isolated from sour yeast a bacillus, which he identified as "Proteus Hauseri," and made numerous experiments on dogs to test its pathogenic power. From five to ten cubic centimetres of a liquefied gelatin culture injected into the circulation, through a vein, caused the typical symptoms of "sepsin poisoning," as formerly described by Bergmann and Schmeideberg (1868). In two dogs which died at the end of forty-eight hours the intestinal tract was found in a condition of intense hemorrhagic infiltration. The spleen and glands of the mesentery were much enlarged. But a bacteriological examination gave an entirely negative result, showing that death resulted from toxæmia and not from septicæmia. Further experiments showed that the dried precipitate obtained from liquefied gelatin cultures, by the addition of alcohol, had the same pathogenic action on dogs, rabbits, and mice as cultures containing the living bacilli. That a similar pathogenic effect is produced in man by the products of growth of this bacillus was shown by the following facts: While conducting his experiments Levy had an opportunity to make a bacteriological examination in the case of a man who died after a brief attack of cholera morbus. From the vomited material and the stools he obtained a pure culture of proteus; but the blood, collected at the autopsy, was sterile. In the mean time seventeen other persons who had eaten at the same restaurant were taken sick in the same way. Upon an examination at the restaurant it was found that the bottom of the ice chest in which the proprietor kept his meats was covered with a slimy, brown layer, which gave off a disagreeable odor. Cultures from this gave the proteus as the principal microörganism present. Levy concludes from his own investigations and those of other bacteriologists that in so-called "flesh-poisoning" bacteria of this group are chiefly at fault, and that the pathogenic effects are due to toxic products evolved during their development.

PROTEUS OF KARLINSKI.

Synonym.—Bacillus murisepticus pleomorphus (Karlinski). Probably identical with Proteus vulgaris of Hauser.

Obtained by Karlinski (1889) from a fibro-purulent uterine discharge, and from abscesses in the uterus and its appendages in a puerperal woman.

Morphology.—Resembles Proteus vulgaris of Hauser in its morphology, and presents various forms under different circumstances relating to the culture medium, the temperature, age of culture, etc.—sometimes as spherical or short oval cells, at others as longer or shorter rods or spiral filaments; usually as bacilli with round ends two and a half times as long as thick, often united in pairs.

Stains with the usual aniline colors, but not by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic, liquefying, motile* bacillus. Spore formation not observed. Grows rapidly in the usual culture media at the room temperature. In *gelatin plate cultures*, at the end of ten hours, small colonies are developed which have well-defined outlines, are oval or whetstone-shaped, of a light-brown color by transmitted light and white by reflected light, with a somewhat darker margin and a smooth surface, sometimes marked by shallow clefts; at the end of twenty hours the colonies commence to have irregular margins, and the surface of the gelatin above them is marked by concentric rings. At the end of thirty hours the colonies have formed a bulb-shaped liquefaction of the gelatin, and delicate, ray-like offshoots are seen around the margin. At the end of two days the bulbous cavities are about one and a half millimetres in diameter and contain a cloudy, grayish-white liquid; they are surrounded by a moist-looking, gray, irregular marginal zone. In *gelatin stab cultures*, at the end of twenty-four hours, a funnel-shaped liquefaction of the gelatin occurs near the surface, and a grayish-white, cloudy mass is developed along the line of puncture; at the end of forty-eight hours a sac-like pouch of liquefied gelatin has formed, and in the course of four or five days the gelatin is entirely liquefied. Upon *agar plates* the colonies are at first oval in form and white by reflected light, or pale brown by transmitted light; at the end of thirty hours the surface becomes wrinkled or folded and is surrounded by radiating, delicately twisted offshoots. Upon the surface of agar a white layer is developed. Upon *potato* a whitish-gray, soft, homogeneous layer, which after standing a long time has a darker color. Upon *blood serum* a thin, grayish-white layer is formed and the serum is rapidly liquefied. Gelatin cultures acquire a strongly alkaline reaction and give off a disagreeable odor resembling that of butyric acid.

Pathogenesis.—White mice inoculated at the root of the tail die in from twenty-two to twenty-four hours; the spleen is greatly enlarged; the bacilli are found in blood from the various organs—less numerous in blood from the heart. Field mice and house mice are less susceptible. Subcutaneous injections in rabbits may give rise to local inflammation and also to general infection. In white rats and guinea-pigs a local abscess may result from a subcutaneous inoculation.

PROTEUS MIRABILIS.

Obtained by Hauser (1885) from putrefying animal substances.

Morphology.—Bacilli resembling very closely the preceding species (Proteus vulgaris), but presenting more numerous involution forms, which may be spherical, pear-shaped, or spermatozoa-like, etc. The bacilli are about 0.6 μ in diameter and vary greatly in length, being sometimes nearly spherical, or forming rods of 2 to 3.75 μ in length, or long filaments.

Biological Characters.—An *aërobic* and *facultative anaërobic, liquefying, motile* bacillus. Spore formation has not been observed. Grows in the usual culture media at the room temperature. Does not liquefy gelatin as rapidly as Proteus vulgaris. Upon *gelatin plates*, at the end of twelve hours, superficial colonies of two to three millimetres in diameter are formed; under a low power these appear finely granular and brownish in color, and have an irregular outline; outgrowths from the margin extend in various directions and form new colonies, which may be attached for a time by a long and slender thread consisting of bacilli. The movement of these new colonies is not as pronounced as in the case of the preceding species, and they are characterized by the presence of numerous distorted bacilli—involution forms. The deep colonies form spiral zoöglœa masses.

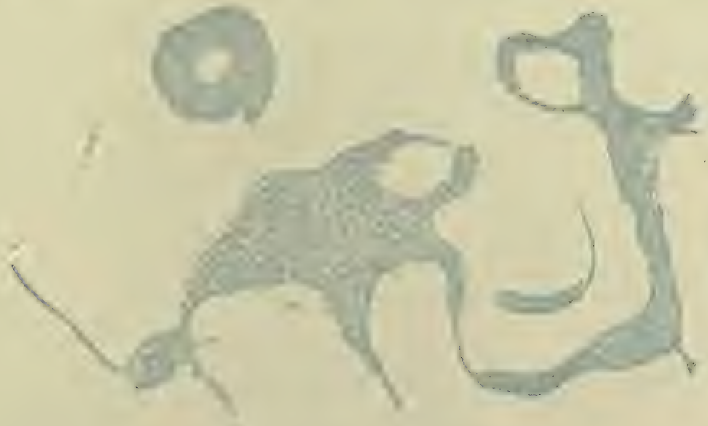

FIG. 155.—"Swarming islands" of Proteus mirabilis, from a gelatin culture. × 285. (Hauser.)

In gelatin stab cultures the whole surface is first covered with threads and islands of bacilli, which after a time form an anastomosing network, and finally a confluent layer which at the end of forty-eight hours is rather thick, with a moist, shining surface and grayish color, and appears to be perforated with numerous small, sieve-like openings. These thinner and transparent places disappear after a time, and at the end of two or three days liquefaction of the gelatin commences; complete liquefaction does not occur until the fifth or sixth day, or even later. Along the line of puncture finely granular colonies are first formed, from which long threads are given off, which form after a short time a tolerably broad zone of threads and spiral zoöglœa masses.

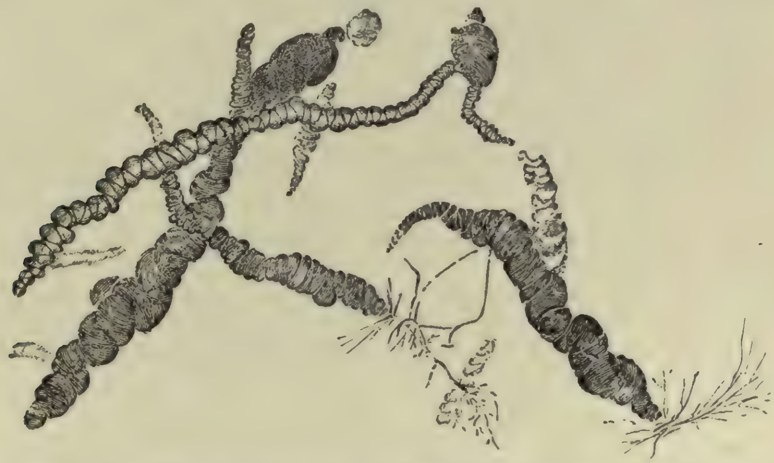

FIG. 156.—Spiral zoöglœa from a culture of Proteus mirabilis. × 95. (Hauser.)

Pathogenesis.—In Hauser's experiments filtered cultures (two to six cubic centimetres), injected into the circulation or into the cavity of the abdomen in rabbits, caused fatal toxæmia.

PROTEUS ZENKERI.

Obtained by Hauser (1885) from putrefying animal substances.

Morphology.—Bacilli which vary greatly in length—average about 1.65 μ, and about 0.4 μ broad.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying, motile* bacillus. Spore formation not observed. Grows in the usual culture media at the room temperature. Upon the surface of nutrient gelatin a laminated mass forms about the point of puncture, from the periphery of which offshoots are given off, at the extremities of which colonies are formed, as in the case of Proteus mirabilis. Gradually a rather thick, grayish-white, opaque layer is formed, which covers the entire surface of the gelatin and is easily detached from it. This species is distinguished from the two preceding by the fact that it does not liquefy gelatin or blood serum and does not give off a decided putrefactive odor when cultivated in these media.

Pathogenesis.—Considerable quantities injected into small animals give rise to local abscesses and to symptoms of toxæmia.

PROTEUS SEPTICUS.

Obtained by Babes (1889) from the mucous membrane of the intestine and the various organs of a boy who died of septicæmia.

Morphology.—Bacilli about 0.4 μ broad and varying greatly in length; slightly curved rods or flexible filaments, often associated in loose chains.

Stains by the usual aniline colors and by Gram's method.

Biological Characters.—An *aërobic, liquefying, motile* bacillus. Spore formation not observed. Grows in the usual culture media at the room temperature. In *gelatin plates* centres of liquefaction are quickly formed and rapidly extend. The spherical, liquefied places have at first a wavy or dentate outline, and are surrounded by a branching, transparent, granular margin which rapidly extends in advance of the liquefaction. In *stab cultures* in nutrient gelatin liquefaction of the entire contents of the tube may take place within twenty-four hours, or a broad, liquefied sac is formed along the line of puncture. Gelatin cultures give off a very disagreeable odor. Upon the surface of *nutrient agar*, at 37° C., a peculiar, thick network extends over the surface in the course of a few hours. Upon *potato* an elevated, brownish-white, shining layer is formed. *Blood serum* is liquefied by this bacillus.

Pathogenesis.—Pathogenic for mice, less so for rabbits. In mice death occurs in from one to three days after the subcutaneous injection of a small quantity of a pure culture; the bacilli are present in the blood in small numbers.

PROTEUS LETHALIS.

Synonym.—Proteus bei Lungengangrän des Menschen (Babes).

Obtained by Babes (1889) from the spleen and gangrenous portions of the lung of a man who died of septicæmia.

Morphology.—Short rods with round ends, from 0.8 to 1.5 μ thick; often swollen in the middle, like a lemon or a flask; forms short, flexible filaments which also present similar swellings.

Stains with the usual aniline colors and also by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying, motile* bacillus. Not observed to form spores. Grows in the usual culture media at the room temperature. In *gelatin plates* forms hemispherical, elevated, whitish, translucent colonies, which later send out coarse branches which ramify over the surface of the gelatin. A similar growth is observed upon the surface of gelatin stab cultures, and an abundant development takes place along the line of puncture. Upon *nutrient agar* a thick, opaque, slightly yellowish layer is formed. Upon *potato* a moist, shining, brownish layer is developed, and the potato acquires a brownish color. Upon *blood serum* the growth is less abundant than on agar; the blood serum is not liquefied. This bacillus grows rapidly at the room temperature; it is destroyed by a temperature of 80° C., and presumably does not form spores.

Pathogenesis.—Recent cultures are very pathogenic for mice and for rabbits, less so for guinea-pigs. The subcutaneous injection of a small quantity of a pure culture kills susceptible animals in two or three days. More or less œdema is found at the point of inoculation. Injections into the rectum of rabbits gave rise to hæmorrhagic enteritis, peritonitis, and death at the end of four days.

BACILLUS A OF BOOKER.

Obtained by Booker (1889) from the alvine discharges of children suffering from cholera infantum.

Morphology.—Bacilli with round ends, varying greatly in length, usually three to four μ long and 0.7 μ broad (in recent agar cultures). In older cultures the bacilli are shorter and smaller.

Biological Characters.—An *aërobic* and *facultative anaërobic*, *liquefying*, *motile* bacillus. Grows at the room temperature in the usual culture media. In *gelatin plates* colonies are visible at the end of twenty-four hours; under the microscope these are nearly colorless, and liquefaction soon occurs around them. In *gelatin stab cultures* complete liquefaction occurs in three or four days. Upon *agar* a colorless layer covering the entire surface is developed in three or four days, and an abundant development occurs along the line of puncture. Agar colonies have a bluish look, and are surrounded by an indistinct halo which shades off gradually into the surrounding agar; under a low power the colonies are light-brown and the borders indistinct; the surface has a delicate, wavy appearance. Upon *potato* the growth is luxuriant and of a dirty-brown color. Blood serum is liquefied by this bacillus.

Milk is coagulated into a gelatinous mass having an alkaline reaction; later the coagulum is dissolved.

Pathogenesis.—Mice and guinea-pigs fed with cultures in milk die in from one to eight days.

BACILLUS ENDOCARDITIDIS GRISEUS.

Obtained by Weichselbaum (1888) from the affected valves in a case of endocarditis recurrens ulcerosa.

Morphology.—Short rods with rounded or somewhat pointed ends, about two to three times as long as broad—of about the same dimensions as the bacillus of typhoid fever.

Stains with the usual aniline colors and also by Gram's method; the longer rods from old cultures are irregularly stained.

Biological Characters.—An *aërobic*, *non-liquefying*, *motile* bacillus. Refractive bodies may be seen in some of the rods, which resemble spores and are stained by the method of Ernst, but they do not show the resistance of known spores to physical and chemical agents. Grows well in the usual culture media at the room temperature. Upon *gelatin plates* colonies are formed which resemble those of Friedländer's bacillus, but which gradually acquire a gray or grayish-white color. The prominent, convex, superficial colonies under a low power are finely granular and grayish-brown in color; the deep colonies are yellowish-brown in color, have slightly notched margins, and the surface is covered with minute projections. In stab cultures a rather thin, circular layer forms about the point of puncture; this has the appearance of stearin; later it becomes grayish-white and the margins are marked by radiating lines. Upon the surface of *nutrient agar* a similar growth occurs which has a pale-brown or reddish-gray color. Upon *potato* in the incubating oven an abundant development occurs, forming a dry-looking layer of a grayish-brown color and having irregularly notched margins. Upon *blood serum* an abundant, grayish-white growth of cream-like consistence forms along the impfstrich; later this has a reddish gray color. This bacillus grows to the bottom of the line of puncture in stick cultures, and is no doubt a facultative anaërobic.

Pathogenesis.—Pathogenic for white mice and for guinea-pigs.

BACILLUS ENDOCARDITIDIS CAPSULATUS.

Obtained by Weichselbaum (1888) from thrombi and embolic infarctions in the spleen and kidneys of a man who died from endocarditis with formation of thrombi.

Morphology.—Resembles Friedländer's bacillus, and is frequently surrounded by a capsule, which may be stained; also forms long, curved filaments, in the protoplasm of which vacuoles may be observed in stained preparations.

Stains with the usual aniline colors, but not by Gram's method; by staining with fuchsin and carefully decolorizing with diluted alcohol the presence of a capsule may be demonstrated.

Biological Characters.—An *aërobic*, *non-liquefying* bacillus. Grows in the usual culture media at the room temperature.

In *gelatin stab cultures* development occurs along the line of puncture, and on the surface as a rather thin, white, dry layer which resembles stearin. In *agar plates* the superficial colonies are thin, about two millimetres in diameter and gray in color; under a low power the margins are transparent and colorless, and the centre resembles the deep colonies; these are very small and grayish-white in color; under a low power the surface is seen to be covered with tooth-like, projecting masses, the margin is dentate and has a pale-yellow color, while the centre is yellowish-brown.

Pathogenesis.—Rabbits are killed by the injection of a considerable quantity of a pure culture into the cavity of the abdomen or subcutaneously.

BACILLUS ALVEI.

Synonym.—Bacillus of foul brood (of bees).

Obtained by Cheshire and Cheyne (1885) from the larvæ in hives infected with "foul brood." The larvæ in the interior of cells in the comb die and become almost fluid as a result of parasitic invasion by this bacillus.

Morphology.—Bacilli with rounded ends, from 2.5 to 5 μ in length (average about 3.6 μ) and 0.8 μ in diameter. Grow out into filaments and form large oval spores which have a greater diameter than the rods in which they are developed—1.07 μ.

Stains readily with the aniline colors usually employed, also by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic*, *liquefying*, *motile* bacillus. Forms endogenous spores. Grows readily in the usual culture media at the room temperature.

In *gelatin plates* small, round or oval colonies are formed, which later become pear-shaped; a branching outgrowth occurs about the margins of the colonies, and especially from the small end of the pear-shaped mass. In streak cultures upon the surface of gelatin growth occurs first along the impfstrich, and from this an outgrowth occurs consisting of bacilli in a single row or in several parallel rows, and forming irregular or circular figures, from which other similar outgrowths occur; the branching outgrowths may anastomose. The gelatin is liquefied in the vicinity of these lines of growth, forming a network of channels. A similar growth is seen upon the surface of *gelatin stab cultures*, and along the line of puncture white, irregular masses are formed, from which rather coarse branches are given off which often have a club-shaped extremity. In older cultures the finer branches disappear, so that the secondary centres of growth are disconnected from the original colonies; complete liquefaction of the gelatin occurs in about two weeks; the liquefied gelatin has a yellowish color and peculiar odor. Upon the surface of nutrient agar, at 37° C., a white layer is formed. Upon *potato* the development is slow and results in the formation of a dry, yellowish layer. In *milk* coagulation first occurs, and the coagulum is subsequently dissolved; a slightly acid reaction is produced. This bacillus grows best in the incubating oven at 37°, and does not develop at temperatures below 16° C. The spores require for their destruction a temperature of 100° C. maintained for four minutes (determined by the writer, 1887).

Pathogenesis.—The introduction of pure cultures of this bacillus into hives occupied by healthy swarms causes them to become infected with foul brood; grown bees also become infected when given food containing the bacillus (Cheshire). Mice injected subcutaneously with a considerable quantity die within twenty-four hours, guinea-pigs in six days (Eisenberg). Small amounts injected beneath the skin of mice or rabbits produce no apparent result.

BACILLUS OF ACNE CONTAGIOSA OF HORSES.

Obtained by Dieckerhoff and Grawitz (1885) from pus and dried scales from the pustules of "acne contagiosa" of horses.

Morphology.—Short rods, straight or slightly bent, 0.2 μ in diameter.

Stains best with an aqueous solution of fuchsin, and also by Gram's method; does not stain well with Löffler's alkaline solution of methylene blue.

Biological Characters.—An *aërobic*, *non-liquefying* bacillus. In *gelatin stab cultures* a very scanty growth occurs along the line of puncture; upon the surface a white mass forms about the point of puncture. Upon *blood serum* and nutrient *agar* an abundant growth at the end of twenty-four hours at 37° C., consisting of white colonies along the impfstrich, which later have a yellowish-gray color. The growth is more abundant and rapid upon blood serum than upon other media.

Pathogenesis.—Pure cultures of the bacillus described are said by Dieckerhoff and Grawitz to produce typical acne pustules when rubbed into the skin of horses, calves, sheep, and dogs. When rubbed into the intact skin of guinea-pigs a phlegmonous erysipelatous inflammation was produced, and the animal died at the end of forty-eight hours with symptoms of toxæmia. Subcutaneous injections in guinea-pigs caused toxæmia and death at the end of twenty-four hours. At the autopsy a hæmorrhagic infiltration of the intestinal mucous membrane was observed; the bacilli were not found in the internal organs. In rabbits pure cultures rubbed into the intact skin caused a development of pustules and a severe inflammation of the subcutaneous connective tissue, from which the animal usually recovered. Subcutaneous injections in rabbits sometimes caused a fatal toxæmia. House mice, field mice, and white mice were not affected by the application of cultures, by rubbing, to the uninjured skin, but succumbed to subcutaneous injections in twenty-four hours or between the fifth and tenth days. Those which died at a late date presented the pathological appearances which characterize pyæmia.

BACILLUS OF PURPURA HÆMORRHAGICA OF TIZZONI AND GIOVANNINI.

Obtained by Tizzoni and Giovannini (1889) from the blood of two children who died of purpura hæmorrhagica following impetigo.

Morphology.—Bacilli with round ends, from 0.75 to 1.3 μ long and 0.2 to 0.4 μ broad; often seen in pairs or in groups like streptococci.

Stains with the usual aniline colors, but not by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying, non-motile* bacillus. Spore formation not observed. Grows in the usual culture media at the room temperature. Upon *gelatin plates* the colonies at first resemble those of Streptococcus pyogenes. Upon the surface small, opaque points are seen at the end of forty-eight hours, which at the end of four to five days develop into spherical, yellowish-gray colonies with irregular margins, surrounded by a growth resembling tufts of curly hair. Upon *agar* the growth is similar, but more rapid and of a pale color, often with a central nucleus surrounded by a net-like marginal zone. Upon *blood serum* the growth is similar to that upon agar. Upon *potato*, at 37° C., a limited development occurs about the point of inoculation, which has a dark-yellow color. The cultures give off a very penetrating odor.

Pathogenesis.—Pathogenic for dogs, rabbits, and guinea-pigs when injected subcutaneously. Not pathogenic for white mice or pigeons. The symptoms resulting from a subcutaneous injection are said to be fever, albuminuria and, in some cases, anuria, hæmorrhagic spots upon the skin, convulsions; death occurs in from one to three days. At the autopsy there are found œdema about the point of inoculation, hæmorrhages in the skin and muscles, and sometimes in the internal organs and in serous cavities; the blood does not coagulate. The bacilli are found in the subcutaneous connective tissue, but not in the blood or in the various organs. Sections show coagulation necrosis of the liver cells and of the renal epithelium.

BACILLUS OF PURPURA HÆMORRHAGICA OF BABES.

Obtained by Babes (1890) from the spleen and lungs of an individual who died from purpura hæmorrhagica with symptoms of septicæmia. Resembles the bacillus previously described by Tizzoni and Giovannini, and still more that of Kolb; but, according to Babes, differs in some respects from both of these, although they all belong evidently to the same group.

Morphology.—Bacilli with rounded ends, oval or pear-shaped, about 0.3 μ thick, surrounded by a narrow capsule.

Stains with the aniline colors, but not deeply, and still less intensely by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying, non-motile* bacillus. Does not form spores. Grows in the usual culture media at the room temperature. In *gelatin stick cultures*, at the end of three days, a thin, transparent, irregular layer has developed upon the surface, and a whitish, punctate stripe along the line of inoculation. In *agar stick cultures* an abundant development occurs along the line of puncture, and at the end of three days the growth upon the surface consists of small, moist, transparent drops; later of larger, flat, shining, yellowish-white plaques which have ill-defined margins. Upon *blood serum* the development is somewhat more abundant in the form of small, white, moist colonies one to two millimetres broad. Upon *potato*, at the end of three days, moist, whitish drops with ill-defined margins.

Pathogenesis.—Inoculations in the conjunctivæ of rabbits produce ecchymoses of the conjunctiva. At the autopsy numerous hæmorrhagic extravasations are found in all the organs, especially in the lungs and liver; the spleen is enlarged; the bacilli can be recovered in pure cultures from the various organs. Old cultures proved to have lost their virulence. Pathogenic for mice, which die from general infection in the course of a few days;

the spleen is enlarged, and hæmorrhages in the serous membranes are usually seen.

BACILLUS OF PURPURA HÆMORRHAGICA OF KOLB.

Obtained by Kolb (1891) from the various organs of three individuals who died in from two to four days from attacks characterized by suddenly developed fever, purpura, and albuminous urine.

Morphology.—Oval bacilli, usually in pairs, 0.8 to 1.5 μ long and 0.8 μ broad, surrounded by a narrow capsule, which is only seen distinctly in preparations from the organs.

Stains with the aniline colors, but not deeply, and still more feebly by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-liquefying non-motile* bacillus. Does not form spores. Grows in the usual culture media at the room temperature. In *gelatin stick cultures*, at the end of four days, a very small, thin, hyaline growth is seen about the point of inoculation. The development is more abundant along the line of puncture. Upon the surface of agar a thin layer is formed with smooth margins. Upon *potato*, at the end of three to four days, a whitish, moist, shining stripe is seen along the impfstrich which is about three millimetres broad.

Pathogenesis.—Injections of 0.5 to 1 cubic centimetre of a bouillon culture into the abdominal cavity of rabbits cause symptoms of general infection in the course of a few days, and not infrequently hæmorrhagic extravasations are seen in the ear muscles. More than one cubic centimetre may cause death in from one to three days. At the autopsy hæmorrhagic extravasations are found in the subcutaneous tissues and in the serous and mucous membranes. The blood has little disposition to coagulate; the bacillus may be recovered in pure cultures from the various organs. In guinea-pigs local ecchymoses are sometimes produced, otherwise not pathogenic for this animal. Pathogenic for mice, which die from general infection, after being inoculated with a small quantity of a pure culture, in from two to three days; spleen enlarged; lymphatic glands often hæmorrhagic. Not fatal to dogs, but animals which were inoculated with one cubic centimetre of a bouillon culture and subsequently killed proved to have hæmorrhagic extravasations in the various organs.

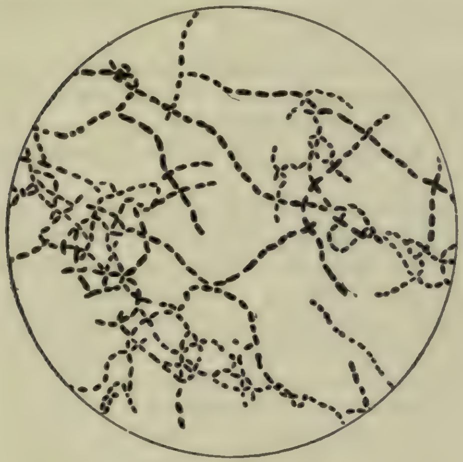

FIG. 157.

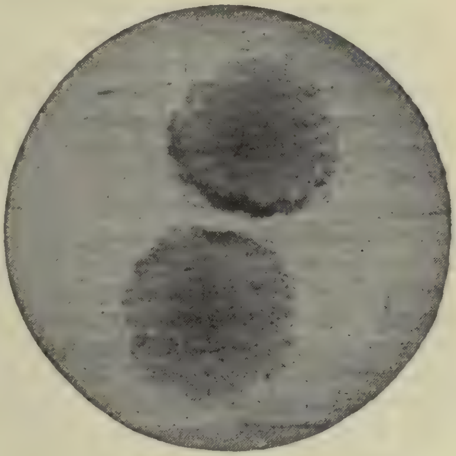

FIG. 158.

FIG. 157.—Bacillus gracilis cadaveris, from a gelatin culture. × 1,000. From a photomicrograph. (Sternberg.)

FIG. 158.—Bacillus gracilis; colonies in gelatin roll tube, end of forty-eight hours. × 12. From a photograph. (Sternberg.)

BACILLUS GRACILIS CADAVERIS (Sternberg).

Obtained (1889) from a fragment of liver, of man, kept for forty-eight hours in an antiseptic wrapping.

Morphology.—Bacilli about 1 μ broad and 2 μ long, associated in long chains.

Biological Characters.—An *aërobic* and *facultative anaërobic*, *non-motile*, *non-liquefying* bacillus. Spore formation not observed. In *gelatin roll-tubes* the deep colonies are opaque and spherical; superficial colonies circular or slightly irregular in outline, white in color, and opaque or slightly translucent. In *gelatin stab cultures*, at 22° C., at the end of five days a rather thick, white mass at the point of puncture, covering one-third of the surface, and closely crowded, opaque colonies at bottom of line of puncture, with slender, branching outgrowth above. In *nutrient agar*, at the end of five days at 22° C., a milk-white growth upon the surface and opaque growth to bottom of line of puncture. On *potato*, at end of five days at 22° C., rather thick, cream-white growth with irregular margins along the impfstrich. Cultures in *bouillon* have a milky opacity and a very disagreeable odor. Grows in *agua coco* without formation of gas.

Pathogenic for rabbits when injected into the cavity of the abdomen.

CAPSULE BACILLUS OF NICOLAIER.

Obtained by Nicolaier (1894) from pus contained in an abscess of the kidney—obtained post-mortem.

Morphology.—Thick bacilli, with rounded ends, usually four times as long as thick, and frequently presenting irregular outlines ; often united in pairs, and sometimes growing out into filaments; cocci-like forms also occur. Often surrounded by a capsule which remains unstained in stained preparations. Does not stain by Gram's method.

Biological Characters.—An *aërobic*, and *facultative anaërobic*, *non-liquefying*, *non-motile* bacillus. Does not form spores. Grows at the room temperature and more rapidly at 37° C. Upon *gelatin plates* at 20° C., at the end of twenty-four to thirty-six hours punctiform colonies are developed, which under a low power appear finely granular, and grayish-yellow spheres. At the end of forty-eight to sixty hours the superficial colonies appear as round or slightly irregular, grayish-white discs, which project but little above the surface of the gelatin, and have thin, transparent margins. The deep colonies have a sharply defined contour, with dark-brown centre and a purely granular pale-brown marginal zone. In *gelatin stab cultures* a slightly elevated, moist-looking, sticky layer with more or less transparent margins is developed. In *slanting cultures* this growth gradually slips down to the lowest part of the exposed surface, leaving a thin, gray, transparent layer over the gelatin ; along the line of puncture a ribbon-like, grayish-white growth with irregular margins is developed. In media containing glucose some gas bubbles are developed. The growth is much more rapid in the incubating oven at 37° C., and there is an abundant development of gas in agar tubes. Upon *potato* a grayish-white, slimy mass with a shining surface is quickly developed. In *bouillon*, at the end of twenty-four hours, at 37° C., the medium is clouded throughout, and a grayish-white deposit accumulates at the bottom of the tube. Development occurs also in acid media.

Pathogenesis.—Pathogenic for house mice, white mice, and for rats—not for rabbits or guinea-pigs—by subcutaneous injections. As Nicolaier has made a careful comparison of the characters of the various "capsule bacilli" described, we quote from him as follows :

"Our bacillus in its morphology and growth in various media closely resembles that of Fasching and of Abel, both of which were obtained in pathological products from man. It is distinguished from them by its pathogenic action upon mice. White and gray mice when infected with our bacillus die from septicæmia and show, in addition to a serous exudation at the point of inoculation, constant pathological changes in the kidneys, which may usually be recognized by a macroscopic examination. Also by the spleen, which is not always enlarged, and the liver, which only in a few cases showed any microscopic changes. In mice inoculated with the bacillus of Fasching, or that of Abel, which died of septicæmia, there was constantly seen an enlargement of the spleen (Fasching, Abel) and of the liver (Abel), and a cloudy swelling of the liver and kidneys (Abel) which our mice failed to show. The macroscopic and microscopic changes which we found in the kidneys in mice, and also in some cases in the liver and spleen, were not observed by Fasching or by Abel. Recently Paulsen has described a capsule bacillus from atrophic rhinitis, and Marchand a capsule bacillus—not further described—which he obtained in great numbers from the exudate in a case of lobar pneumonia. Both appear to be very similar to Fasching's bacillus. They are pathogenic for mice, but do not cause the changes in the kidneys which we have described. These capsule bacilli are therefore not identical with ours. Marchand's bacillus is further distinguished by the fact that it is pathogenic for guinea-pigs. . . . The bacillus of Kockel is distinguished from ours by the following characters: It forms upon the surface of gelatin, as well as in stick cultures, highly elevated, button-like colonies, while our bacillus grows more in flat and broad layers. It also lacks the semi-fluid character of growth upon slanting agar, which distinguishes our bacillus, and as a result of which the growth slips down to the lowest point on the slanting surface; further it forms upon potato a yellowish layer, while ours is grayish-white; and it does not grow in acid media. Finally, it is pathogenic for rabbits by intravenous injection, while ours is not."

BACILLUS MUCOSUS OZÆNÆ.

Obtained by Abel (1893) from cases of ozæna simplex (rhinitis atrophicans fœtida). As this bacillus appears to correspond in its morphological and biological characters with the capsule bacillus above described we shall not repeat this description, but quote from Abel, as follows:

"This bacillus, found in the secretion from cases of ozæna, as the description we have given shows, closely resembles Friedländer's pneumobacillus. It is distinguished from it by certain constant characters. The ozæna bacillus forms in cultures a more fluid mass than Friedländer's. As a result of this it does not form the characteristic nail-head culture, but spreads out over the surface of the gelatin. Upon slanting gelatin cultures the growth slips down to the lowest point. In old cultures it never shows a brown coloring of the culture medium. It never forms gas on potato, and in agar and gelatin cultures but little gas is developed. Mice always succumb to subcutaneous inoculations, while Friedländer's bacillus does not kill mice. Intraperitoneal infection of guinea-pigs with the ozæna bacillus always causes their death. Friedländer's bacillus only killed about half the guinea-pigs inoculated in the cavity of the abdomen. Finally, Friedländer's bacillus has a greater tendency to cocci-like forms. The resemblance to Pfeiffer's capsule bacillus is closer. But the tenacious layer described by Pfeiffer as found upon the intestinal coils and the lungs in mice, and the sticky condition of the blood and tissue juices (*fadenziehende*) are wanting. The reaction at the point of inoculation in mice is also much more pronounced with my bacillus."

It seems extremely probable that this bacillus, the Bacillus capsulatus mucosus of Fasching, and the above-described capsule bacillus of Nicolaier are simply pathogenic varieties of one and the same bacillus.

CAPSULE BACILLUS OF VON DUNGERN.

Obtained by von Dungern (1893), post mortem, from a new-born child which died of hemorrhagic septicæmia—infection through umbilicus.

Morphology.—A short, thick bacillus, from 1 to 2 μ long and half as broad, surrounded by a capsule which is slightly stained by gentian violet—best seen in the body of infected mice; sometimes seen in pairs or in chains of four elements; also grows out into filaments, especially in bouillon. Upon potato usually only small spherical elements, resembling micrococci, are seen. Does not stain by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic*, *non-motile*, *non-liquefying* bacillus. Does not form spores. Coagulates *milk*, in which it causes an abundant development of gas at 38° C. Has feeble indol reaction. Grows well at room temperature, more rapidly in incubator. Upon *gelatin plates* the deep colonies at end of twelve hours are the size of a pin's head, finely granular, spherical, and sharply defined. Upon the surface, porcelain-like, elevated, white colonies are developed, which in two or three days attain the size of lentils. In *gelatin stab cultures* development occurs all along the line of puncture, frequently with formation of gas bubbles. Upon *agar* a thick, soft layer of a white color is developed. In bouillon, at 38° C., there is considerable development of gas. Upon *potato* the growth is very abundant, of a pale yellowish-white color, thick, soft, somewhat sticky, and filled with gas bubbles. A great portion of the surface is covered by this growth at the end of twenty-four hours, even at the room temperature. These cultures give off a peculiar odor, sometimes aromatic-fœtid and sometimes recalling that of fresh bread. Some of the cultures on potato soon become cream-like in consistence. At first they have an alkaline and later an acid reaction, when they have the odor of acetic acid.

Pathogenesis.—Very pathogenic for white mice. The bacilli are found in the blood and in all the organs in enormous numbers. At the point of inoculation there is frequently a hemorrhagic œdema. The spleen is greatly enlarged. Also pathogenic for guinea-pigs when injected into the cavity of the abdomen—less pathogenic for rabbits.

According to von Dungern, this bacillus can not be distinguished by its morphological and biological characters from Friedländer's bacillus, Bacillus capsulatus of Pfeiffer, or Bacillus canalis capsulatus of Mori. But it is distinguished from these by greater virulence, especially for rabbits, and by the fact that it frequently gives rise to hemorrhagic extravasations in inoculated animals. In our opinion the characters given do not justify the view that this bacillus is a distinct species from the bacilli above mentioned.

BACILLUS PESTIS (Kitasato and Yersin).

Discovered by Kitasato (1894) in the blood of living patients, and in the buboes, blood, and organs of those who had recently died from the infectious malady known as bubonic plague. Kitasato was sent to Hong-Kong by the Japanese Government for the purpose of investigating this disease. According to Lowson the bacilli are found in the fæces, in the contents of the buboes, and in the blood.

Morphology.—In his preliminary note, Kitasato described the plague bacilli as "rods with rounded ends," which are readily stained by the ordinary aniline dyes, the poles being stained darker than the middle part, especially in blood preparations, and presenting a capsule sometimes well marked, sometimes indistinct.

Yersin, who was sent by the French Government to study the bubonic plague at Hong-Kong, arrived in that city on the 15th of June, 1894. He describes the bacillus found in the contents of the buboes as being short and thick, with rounded ends, staining easily with the aniline colors, but not by Gram's method. "The extremities stain more intensely than the centre, so that they often present a clear space in the middle. Sometimes the bacilli appear to be surrounded by a capsule. . . . In bouillon the bacillus has a very characteristic appearance, resembling the cultures of the streptococcus of erysipelas—a clear liquid with grumous deposits on the walls and at the bottom of the tube. These cultures examined under the microscope show veritable chains of short bacilli, presenting in places a considerable spherical enlargement."

This bacillus is sometimes seen to be motile, and it has flagella, which, however, are difficult to stain (Gordon).

In agar cultures, in the incubator at 37° C., involution forms soon appear. These may be spherical, oval, pyriform, etc., and are often many times larger than the typical bacillus.

Biological Characters.—We quote from Kitasato's preliminary report as follows:

> The bacilli show very little movement, and those grown in the incubator, in beef-tea, make the medium somewhat cloudy. The growth of the bacilli is strongest on blood serum at the normal temperature of the human body (34° C.); under these conditions they develop luxuriantly and form a colony moist in consistence and of a yellowish-gray color; they do not liquefy the serum. On agar-agar jelly (the best is good glycerin agar) they also grow freely. The different colonies are of a whitish-gray color and by reflected light have a bluish appearance; under the microscope they appear moist and in rounded patches with uneven edges; at first they appear everywhere as if piled up with "glass-wool," later as if having dense, large centres. If a cover-glass preparation is made from a cultivation on agar-agar, and, after having been stained, is observed under the microscope, long threads of bacilli are seen, which might, by careless inspection, be mistaken for a coccus chain, but are recognized with certainty as "threads of bacilli" under closer observation. The growth on agar-gelatin is similar to that on agar-agar; in a puncture cultivation at the ordinary temperature after a few days they are found growing as a fine dust in little points alongside the puncture, but with very little growth on the surface. Whether these bacilli are able to liquefy ordinary gelatin or not I am at present unable to decide, as the temperature of Hong-Kong ranges so high that the employment of simple nutritive gelatin is out of the question. I shall give further information on this question later. On potatoes at a temperature of from 28° to 30° C., there was no growth after ten days' observation, but at a temperature of 37° C. the bacilli developed sparingly after a few days; the growth was whitish-gray in color and exsiccated. As mentioned before, the bacilli grow best at a temperature of from 38° to 39° C.; at how low a temperature growth is possible I am unable at present to state. So far I have been unable to observe the formation of spores.
>
> *Experiments on Animals.*—Mice, rats, guinea-pigs, and rabbits are susceptible to inoculation. If these animals are inoculated with pure cultivations, or with the blood of a plague patient in which the bacilli have been

observed, or with the contents of a bubo, or with pieces of internal organs, or even with the contents of the intestine, they begin to become ill in from one to two days, according to the size of the animal. Their eyes become watery, they begin to show disinclination for any effort, later they avoid their food, and hide quietly in a corner of the cage. The temperature rises to 41.5° C., and with convulsive symptoms they die in from two to five days. I must observe that in Hong-Kong I could only obtain small guinea-pigs (weight from one hundred to one hundred and fifty grammes) and small rabbits (from two hundred to two hundred and fifty grammes). If I could have experimented upon larger animals it is possible that life would have been prolonged somewhat beyond the periods mentioned above. The parts around the point of inoculation are infiltrated with a reddish gelatinous exudation, the spleen is enlarged, sometimes there is a swelling of the lymphatic glands, and in all the organs the bacilli are found. The results found after death in animals are very similar to those found in anthrax and in œdema malignum. Pigeons do not appear to be susceptible to the influence of the bacilli. I made experiments by feeding some mice and guinea-pigs with pure cultivations of the bacillus and with small pieces of the internal organs: the result was, such animals perished in a few days under the same symptoms as those which had been inoculated. In all the internal organs of animals so destroyed I found the bacilli. With the dust of dwelling-houses from which the plague-stricken had been removed, I made several experiments upon animals. Some of the animals died from tetanus. In one case only a guinea-pig died with plague symptoms, and in this animal the same bacilli were found in the internal organs as in those of plague patients who had succumbed. These experiments with the dust from infected houses I shall certainly continue. Many rats and mice at present die spontaneously in Hong-Kong. I examined some of them. In the internal organs of a mouse I discovered the same bacilli.

Experiments with Desiccation.—The contents of a bubo in which the bacilli were present in great numbers were wiped over cover glasses (perfectly cleansed by heat and alcohol), and some of these cover-glasses were dried in the air of a room at a temperature ranging from 28° to 30° C. Others I exposed directly to the sun's rays, and from among them, after an exposure of from one, two, and three hours up to six days, I removed some parts, putting such portions in beef-tea and placing them in the incubator. Those which had been standing in the room from one to thirty-six hours showed a pretty good growth in the incubator, but those which had been in the room for more than four days were unable to show any growth even after one week's incubation. Those exposed directly to the sun were all destroyed after from three to four hours. Further cultivations on serum were treated exactly like the contents of the bubo with very similar results.

Experiments with Heat.—Beef-tea cultivations which had been heated for thirty minutes in a water bath up to 80° C. were destroyed; at 100° C., in the vapor apparatus they were destroyed in a few minutes.

Yersin reports that when fragments of the spleen or liver of animals which have died of the plague are fed to rats and mice they usually become infected and die, and the bacillus is found in their organs, lymphatic glands, and blood. He also demonstrated the presence of the bacilli in dead rats found in the houses or streets of Hong-Kong.

Without doubt rats play an important part in the propagation of the disease. Monkeys are also very susceptible to infection, and it is said that the disease has been known to occur as an epidemic among these animals. There is also good reason to believe that fleas have some influence in the propagation of the disease, by transferring the bacillus from infected rats to man, or from one individual to another. Infection in man occurs by inoculation through lesions of the skin and also by the respiratory passages (pulmonic form).

BACILLUS PISCICIDUS AGILIS (Sieber).

Discovered by Sieber (1895) in infected fish, which died of an epidemic disease in the laboratory of Professor Nencki, at St. Petersburg.

Morphology.—Short bacilli, often united in pairs.

Biological Characters.—An *aërobic* and *facultative anaërobic*, *motile*, *liquefying* bacillus. In old cultures in bouillon spores are developed. Grows at temperatures of from 12° to 37.5° C. Thermal death point, 60° to 65° C. On *gelatin and agar plates* forms granular, grayish, or yellowish colonies, which appear to be made up of three concentric rings—the outer one having a jagged outline. Gas is developed during the growth of the bacillus—carbon dioxide and methyl merecaptan in small amount. Upon *potato* it forms yellowish-brown, pearl-like colonies. Causes coagulation of milk. Retains its vitality and virulence for months in well or river water.

Pathogenesis.—Pathogenic for fish, frogs, guinea-pigs, rabbits, mice, and dogs (not for birds). Old cultures are more pathogenic than recent ones, and gelatin cultures are the most active. Frogs are killed in half an hour by 0.1 cubic centimetre of a bouillon culture six days old. Filtered cultures are as toxic as those containing the living bacillus; they give with iron chloride a characteristic color reaction—an intense red color. Sieber has obtained from his cultures an extremely toxic alkaloid in the form of a hydrochlorate. Two litres of filtered culture gave 0.1 gramme of this salt. An aqueous solution of this killed a frog in fifteen minutes in the dose of 0.0035 gramme.

BACILLUS OF MERESHKOWSKY.

Obtained by Mereshkowsky (1894) from infected animals (Spermophilus musicus) which died from an epidemic malady developed in his laboratory.

Morphology.—Closely resembles Löffler's *Bacillus typhi murium.*

Biological Characters.—An *aërobic*, *motile*, *non-liquefying* bacillus. Spore formation not observed. Grows in the usual culture media at the room temperature—best at 37.5° C. In bouillon, at the end of twenty-four hours, the medium is clouded and a white pellicle is seen upon the surface, which breaks up into small flocculi and falls to the bottom when the tube is slightly shaken. On gelatin plates minute, slightly granular, pale-brown colonies may be seen, under a low power at the end of twenty-four hours; on the second day these are visible as white spheres, which under the microscope have a pale-brown color and a more or less transparent, peripheral zone. In media containing glucose no gas is developed. The growth upon agar and potato presents nothing characteristic.

Pathogenesis.—Pathogenic for *Zieselmausen* (Spermophilus musicus), for Spermophilus guttatus, for squirrels (Sciurus vulgaris) for house mice, for field mice (Arvicola arvalis). Not pathogenic for man or for the domestic animals tested, horse, swine, sheep, fowls. Mereshkowsky proposes to use cultures of this bacillus for the extermination of field mice, which die in from one to ten days after being fed upon biscuit wet with a bouillon culture.

BACILLUS OF EMMERICH AND WEIBEL.

Obtained by Emmerich and Weibel (1894) from infected trout in ponds belonging to an establishment for raising these fish. The disease appeared as a superficial "furunculosis with secondary development of abscesses containing bloody pus." Death occurred in from twelve to twenty days. The pustules and secondary abscesses and blood from the heart and various organs contained bacilli, which proved to be the cause of the infectious malady.

Morphology.—Bacilli about as long as the typhoid bacillus, but not so thick, very frequently united in pairs; occasionally grows out into filaments.

Biological Characters.—An *aërobic* and *facultative anaërobic, liquefying, non-motile* bacillus. Does not form spores. Thermal death point, 60° C. Stains with the usual aniline colors but not by Gram's method. Grows best at 10° to 15° C. The growth in gelatin is quite characteristic. At the end of two or three days, in *gelatin plates*, at the room temperature, small white colonies are developed; in four or five days small gas bubbles or excavations are seen, at the bottom of which lie the scale-like or rosetta-formed colonies. The margin of the colonies is irregular and later jagged. At first the colonies are grayish-white or yellowish, later brownish. The superficial colonies have a peculiar lustre. In *gelatin stab cultures*, colonies develop along the line of puncture, which at first resemble the growth of Streptococcus pyogenes, and no development is seen on the surface. At the end of five to seven days in place of the line of colonies is seen a channel filled with air, or gas developed by the separate colonies, the bubbles from which coalesce. The funnel formed in this way is somewhat larger above, and at the bottom contains a whitish sediment consisting of bacteria contained in a few drops of liquefied gelatin. Along the sides of the funnel bubble-like cavities may frequently be seen, at the bottom of which the bacteria have accumulated. In *bouillon* a slight cloudiness is seen near the surface, on the walls of the test tube; when slightly shaken this falls to the bottom, leaving the bouillon entirely clear. In *agar-agar* tubes, a veil-like stripe develops along the line of puncture, and a grayish-yellow, moist layer, with irregular outlines upon the surface. After some weeks this acquires a brown color. No growth occurs upon potato. No development occurs in the incubating oven at 37° C.

Pathogenesis.—Trout became infected and died through direct infection, subcutaneous or intramuscular inoculations, or through the addition of cultures to the water in which they were kept, or by placing infected fish in the same tank with healthy ones.

BACILLUS OF BECK.

Synonym.—Der Bacillus der Brustseuche beim Kaninchen.

Obtained by Beck (1892) from rabbits which died of an infectious malady in the Institut für Infectionskrankheiten, in Berlin.

Morphology.—Very small and slender bacilli, about twice as long and twice as thick as the influenza bacillus; somewhat pointed at the extremities; show a tendency to grow out into filaments.

Biological Characters.—An *aërobic* (strict) *non-liquefying, non-motile* bacillus. Spore formation not observed. Grows at the room-temperature and more vigorously at 38° C. Does not stain by Gram's method. Thermal death point, 50° C. (five minutes). Resists desiccation, at the room temperature, for seventeen days, at 37° C. for three days.

On *gelatin plates*, at the end of forty-eight hours, small, finely granular, glass-like colonies are developed; older colonies have a pale-brown appearance. In gelatin stab cultures a granular growth of a white color is seen along the line of puncture. Upon *agar*, at 37° C., an abundant development occurs in twenty-four hours. The line of puncture seen from above is grayish-white, by transmitted light bluish and porcelain-like with a brownish tint. On agar plates the colonies have a yellowish-gray appearance; the margin of the finely granular colonies is sharply defined. In agar cultures several days old the colonies are sticky and may be picked up as a compact mass, or drawn out into threads. In *bouillon*, at 37° C., there is a slight cloudiness at the end of twenty-four hours; later the bouillon is clear and a white sediment is seen at the bottom of the tube. In bouillon cultures especially, the bacillus grows out into long filaments.

Pathogenesis.—From 0.25 to 1 cubic centimetre of a bouillon culture injected into the pleural cavity of a rabbit caused a development of all of the symptoms of influenza (Brustseuche)—viz., elevation of temperature at the end of five or six hours, cough, nasal discharge, dyspnœa, and death—usually in from three to five days. The autopsy showed a distinct pleuropneumonia and a general blood infection by the bacillus in question. Injections into the circulation also give rise to the symptoms of influenza, including pneumonia, and to death at the end of from ten to fourteen days. Subcutaneous injections resulted in the development of an abscess and of extensive necrosis of the tissues, but did not cause a general blood infection. Guinea-pigs were somewhat less susceptible than rabbits, but injections into the pleural cavity produced similar symptoms and death at a later date. White mice and house mice, as a result of intraperitoneal injections, died within two or three days from general blood infection.

BACILLUS PISCICIDUS (Fischel and Enoch).

Obtained by Fischel and Enoch (1892) from an infected carp.

Morphology.—Bacilli solitary or in chains of four to five elements, 1.2 to $3\,\mu$ long and $0.25\,\mu$ thick. Stains by the usual aniline colors and by Gram's method.

Biological Characters.—An *aërobic* and *facultative anaërobic, non-motile, liquefying* bacillus. Forms spores. In *gelatin* plates forms round colonies of a pale yellowish-brown color, having a slightly toothed border and a granular surface. At the end of twenty-four hours a narrow zone of liquefaction can be discerned around the colonies, and at the end of about ten days the gelatin is entirely liquefied. In gelatin stab cultures a scanty growth is seen along the line of inoculation at the end of twelve hours; the growth upon the surface is rapid, and liquefaction commences at the end of twenty-four hours. Upon *agar*, at 37° C. at the end of eighteen hours, a thin granular layer is seen, which consists of small, pale-gray colonies. In agar stick cultures a scanty growth occurs along the line of puncture, which does not increase after thirty-six hours. Upon the surface the growth is abundant, forming, at the end of five days a tolerably thick grayish-white layer. No growth occurs upon *potato* at the room temperature, but at 37° C. a tolerably thick, sticky layer of a grayish-white color is developed in three or four days. In *bouillon*, at 37° C., the medium is clouded at the end of twelve hours, and a thin pellicle is seen upon the surface at the end of thirty-six hours; this falls to the bottom when the tube is slightly agitated. At the end of four days development has ceased, and the bouillon is again transparent, while a flocculent deposit is seen at the bottom of the tube. The bouillon gives off a penetrating odor, like that of burnt milk. The same odor is given off from cultures in *milk*, which is peptonized by the action of the bacillus. At the end of twenty days, at 37° C., the entire contents of the tube have become transparent.

Pathogenesis.—Produces a fatal infectious disease in fish ("gold carp") when inoculated beneath the skin; also pathogenic for mice and for guinea-pigs.

BACILLUS PYOGENES FILIFORMIS (Flexner).

Obtained by Flexner (1895) from the interior of the uterus and from an exudate in the pericardial and pleural cavities, of a rabbit which died on the fifth day after parturition.

Morphology.—Pleomorphous cocci-like forms, short or long bacilli, and long threads are seen in cover slips prepared from the exudate. "Very few of the bacilli stain regularly; for the most part brightly stained spots appear between stained areas. An outer membrane always stains, enclosing the stained dots in a colorless ground. The threads, as a rule, present delicate, sinuous, and wavy outlines; the short forms are straight with rounded ends."

Biological Characters.—All attempts to cultivate this bacillus in the usual media, either in the presence of oxygen or in an atmosphere of hydrogen, proved unsuccessful. But successive cultures were made by inoculations in the pleural cavity of rabbits—a bit of pleural exudate suspended in bouillon was used for this purpose. The bacillus was also propagated upon the lungs, heart, uterus, and kidney of healthy rabbits. The organs were removed with great care to prevent contamination and placed in sterilized test tubes. Transplantations from these cultures were only successful for one or two generations. Better results were obtained by cultivating the bacillus upon the one-third to one-half grown fœtuses of rabbits.

Pathogenesis.—"Considerable variations were observed according as the inoculations were made into the pleural cavity, the peritoneal cavity, the subcutaneous tissue, beneath the dura mater, or directly into the circulation. The inoculations gave positive results in all cases except a few, in which they were made subcutaneously. The death of the animal occurred soonest when inoculation was made beneath the dura mater. A small portion of the skull was trephined, care being taken to exclude extraneous microörganisms, and a drop of the pleural fluid or a speck of the fibrinous exudate was introduced beneath the membranes, care being taken not to injure the brain. These animals, which quickly recovered from the effects of the operation, died on an average about twelve hours after the inoculation. . . .

"The pleural inoculations were followed by death, as before stated, in every instance, the death of the animal occurring upon the third or fourth day. The appearances presented at the autopsy were for the most part an exact reproduction of those observed in the animal which had succumbed to the natural disease. Upon the side of inoculation a thick, grayish-yellow, shaggy membrane covered the pleural surfaces, being at times four or five millimetres in thickness. The pleural cavity contained several cubic centimetres of a clear hæmoglobin-colored fluid, the lung for the most part being compressed. At times smaller or larger areas of lobular pneumonia would be present; and, as a rule, the inflammation was not limited to the serous membrane of the side of inoculation, but extended into the opposite pleural cavity and into the pericardial sac. However, in these situations the process was, as a rule, less intense, the solid exudate being less considerable, and in the case of the opposite pleural cavity sometimes entirely wanting. The superficial vessels, however, were injected and the serous surface of the affected membrane covered with a slimy, clear fluid. In addition to this the opposite pleural cavity always contained a similar pink serum to that described upon the side of inoculation.

"The study of the exudate upon the side of inoculation as well as the fluid contained in the opposite pleural cavity and in the pericardium showed the same organisms as had been introduced."

BACILLUS DYSENTERIÆ.

The researches of Shiga, of Flexner, and of the board of medical officers of the army engaged in the study of tropical diseases in the Philippine Islands (1890) give support to the belief that there is a form of acute dysentery which is due to infection by the bacillus of Shiga, which Flexner describes as follows:[1]

"Bacillus of the average size of B. coli communis. There is variation in length: almost none in thickness. The individuals are usually separate;

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[1] Johns Hopkins Hospital Bulletin, vol. xi., No. 115.

sometimes they are united in pairs, but only very rarely do they occur as filaments. The ends are slightly rounded. The bacillus shows moderate motility; Gram's stain is negative.

"Growth takes place upon all culture media at the room temperature, but better in the thermostat. Gelatin is not liquefied. The colonies resemble those of B. typhosus, being more nearly like them when first isolated from the dejecta than after a period of cultivation outside the body. After many months of such saprophytic growth the colonies become thicker, exhibit a moist surface, and are less translucent. The strokes upon agar slants show a similar alteration. At first the growth extends but little laterally, but later on it becomes two to three millimetres in width, and generally shows distinct indentations at the edges. Upon gelatin the colonies are more delicate; the stab extends along the line of puncture only, spreading very little at the surface of the medium.

"On potato, growth takes place along the line of inoculation and spreads beyond. After some days it is a little elevated and of a pale-brown tint. On unfavorable potatoes the growth is slight, moist, and membranous, resembling, except for the greater amount of moisture, that of B. typhosus when typical.

"Sugars—glucose, lactose, and saccharose—are not fermented gaseously. In glucose media a moderate acid production takes place.

"Bouillon is clouded diffusely and a sediment forms. There is no production of a pellicle.

"Litmus milk assumes, after twenty-four to seventy-two hours, a faint lilac tinge. After the lapse of from six to eight days alkali begins to be produced, which increases in amount until the litmus is rendered deep blue in color. No coagulation of the milk ensues.

"Indol is not always formed. Even in sugar-free bouillon it may fail to appear, or it may be produced in small quantities only.

"Suitable cultures of this organism, when tested for the agglutination reaction with the blood serum of persons suffering from dysentery—the host of another individual—give, in many cases, a positive result.

"The bacillus is pathogenic for the ordinary laboratory animals. It is abundant in the acute cases in which it may be the predominating organism; it becomes more difficult to find as the cases progress toward recovery or chronicity. In the ordinary chronic dysentery of Manila, in which amœbæ are commonly encountered, it was not found. It can be cultivated from the dejecta during life, and the intestinal contents, mucous membrane, and mesenteric glands in fatal cases.

"Since the publication of Shiga's studies, Escherich and Celli have both attempted to show that the organisms obtained from their respective epidemics of dysentery are identical with the B. dysenteriæ. In both cases they have proceeded upon the false assumption that Shiga's microörganism was a variety of B. coli communis, whereas, in point of fact, it is much more nearly related in its cultural and physiological properties to B. typhosus.

"The question naturally arises, In what ways does it differ from B. typhosus? Comparison of the Eberth-Gaffky and Shiga bacilli show the criteria of difference to be by no means numerous. The main features, however, are as follows: The latter shows less marked motility when first isolated and a tendency to lose motility rapidly in artificial cultivations; it displays a more uniform generation of indol; after a brief preliminary acid production in milk it gives rise to a gradually increasing alkalinization; it is inactive to blood serum from typhoid cases; but reacts with serum from dysenteric cases to which B. typhosus does not respond. . . .

"Bearing directly upon these considerations are the results of Lieutenant Strong's studies, continued after our departure from Manila. He writes: 'After you left we had a large number of acute cases of dysentery. It seems certain that this form, which we have begun to speak of as *acute infectious dysentery*, is independent of amœbæ. I have now records of fourteen cases (not all were fatal) which I studied bacteriologically. From the stools in all of these, there has been obtained a bacillus which agrees with the organism obtained by you. I have also obtained the organisms from the mesenteric glands in three fatal cases. In one case of acute dysentery with secondary acute fibrinous peritonitis I obtained it from the exudate. The agglutination reaction is not invariable. Amœbæ were never demonstrable in any of these fourteen cases. On the other hand, in every case with certain anatomical lesions we always find the amœbæ. In some cases of dysentery in which the amœbæ were absent and the bacilli present, that have lasted four to five weeks (one case lasted nearly two months) and then resulted fatally, we see a continuation of the same process that is observed in the acute fatal cases. The lesions are those of necroses of the mucous membrane and induration of the gut.' "

XV.

BACTERIA OF PLANT DISEASES.

I SHALL not attempt to give a full account of the bacteria which have been described as bearing an etiological relation to various infectious diseases of plants, but a "text-book of bacteriology" would be incomplete without some reference to the best known of these bacteria. In the following descriptions of species I have preferred to quote largely from the published papers of Dr. Erwin F. Smith, of the Department of Agriculture, United States, a recognized authority in the investigation of plant diseases, rather than to rewrite his descriptions.

BACILLUS SOLANACEARUM (Smith).

Causes a bacterial disease of the tomato, egg plant, and Irish potato.

"*Morphology.*—A medium-sized bacillus, with rounded ends; often in pairs, with a plain constriction; elliptical, but of variable size, depending on age of culture or the length of time the tissues of the plant have been occupied; usually one and one-half to three times as long as broad. On cover-glass preparations made from peptone beef bouillon cultures forty-eight hours old and stained with a watery solution of methyl violet, many are 1.5 by 0.5 μ, but these measurements must not be taken too literally, since the size depends not only on the age of the culture but also on the kind of stain employed, *i.e.*, on whether or not the cell wall stains. Organism motile, often only sluggishly so, especially when taken from the plant, but sometimes very actively motile, especially in young cultures. Flagella much longer than the rod; several—exact number and place of attachment not made out clearly, owing to imperfect preparations (Van Ermengem's method), but apparently arising from any part of the rod. An attempt to stain them by Löffler's method was unsuccessful. No spores observed either in the plant or in culture media, but the search has not been continued long enough to warrant any opinion as to their existence. Zoöglœa are formed almost from the start in fluid culture media.

"*Symptoms Produced in the Plant.*—The first indication of this disease, or at least the first one to attract the farmer's attention, is the sudden wilting of the foliage. This may occur first on a single shoot, but finally it affects the whole plant. Subsequently, and especially if the plant is young or not very woody, the stem shrivels, first changing to a yellowish-green or to a muddy green, and finally to brown or black. The vascular bundles become brown long before the shrivelling takes place, and in the potato often show through the outer green parts of the stem as long, dark streaks, or the bacteria run out on the petioles, after the manner of pear blight, forming narrow, black lines. The vessels of such bundles are filled with the bacilli, which ooze out when the stem is cut across. The foliage may wilt with or without a preliminary yellowing. If the bacteria are abundant in the vessels of the stem, the wilt is often very sudden and the foliage has no time to become yellow. The progress of the disease seems to be more rapid in young than in old plants and in hot than in cold weather.

"In the case of the potato the tubers are also finally attacked and destroyed, the organism reaching them by way of the vascular bundles of the stem. A brown or black rot ensues, beginning in the stem end of the tuber in the vascular ring and extending in all directions therefrom. All stages of this rot of the tubers (both in 1895 and in 1896) were obtained repeatedly from pure cultures of the bacillus pricked into the stem several feet above ground.

"*Bouillon and Peptone Cultures.*—This organism grows well at room temperatures of 20° to 30° C., in beef broth peptonized (Witte's peptonum siccum). It seemed to make little difference whether the bouillon was left acid or rendered slightly alkaline with carbonate of soda. The gathering of the zoöglœa in the upper layers of the fluid is very distinct, especially if the tubes are left undisturbed in an upright position for some days. On shaking the turbidity becomes uniform. The organism produces a copious, dirty white precipitate (much more precipitate than *B. tracheiphilus*).

"The inoculated tubes of *litmus milk* developed no acid—*i.e.*, showed no trace of reddening. After two or three days the litmus became perceptibly bluer than in the control tubes, and this bluing increased from day to day, indicating a progressing alkalinity. This change took place at room temperatures of 20° to 30° C., and also in the thermostat at 37° C. The casein was not precipitated.

"*Gelatin.*—In plate cultures of nutrient gelatin the buried colonies are circular in outline (globose), yellowish or brownish, granular (under Zeiss sixteen millimetres objective and 12 compensating ocular), and with well-defined margins. No oblong or spindle-shaped colonies could be found. The circular outline and regularity and distinctness of the margin of the colony were especially noteworthy. Whether these features will be found constant with all gelatins is a question yet to be determined. Occasionally, after a few days, a narrow, clear zone appeared around the margin of many of these colonies as if liquefaction had set in. This, however, did not progress, or increased but very slowly, and was clearly visible only under the compound microscope. The buried colonies remained small, as if requiring more oxygen than they were able to get. The surface colonies were circular, thin, thin-edged, smooth, white, and wet-shining. They did not spread over the plate rapidly or cause any liquefaction (fifteen per cent gelatin, temperature 20° to 27° C.).

"The organism grew best in a gelatin of the following composition: Lean minced beef, five hundred cubic centimetres; distilled water, one thousand cubic centimetres; mixed and set twenty-four hours in a cool place; filtered and added ten grammes of Witte's peptonum siccum and one hundred and fifty grammes of L. and F. gelatin. This gelatin was clarified with egg and rendered alkaline with sodium hydrate, titrating with phenolphthalein. The degree of alkalinity was between twelve and fourteen of Mr. Fuller's scale.

"*Agar.*—In poured plates of nutrient agar the buried colonies differed considerably from those in gelatin. Instead of being circular with a very smooth margin, they were irregularly round or even oblong, with a decidedly irregular granular margin. These colonies were brown or yellowish-brown under sixteen millimetres objective and 12 ocular. After some weeks the whole body of the agar became decidedly brown. No spindle-shaped colonies were to be seen The surface colonies grew rather slowly. They were dirty-white, smooth, wet-shining, and did not spread widely over the agar.

"The behavior on *potato* is very characteristic. In twenty-four to forty-eight hours (temperature 27° to 32° C.) the fluid became turbid and the projecting part of the cylinder was covered with a copious, wet-shining growth. At first this growth was white or dirty white, but after some days (three to ten) it became brown, and finally, in places, nearly or quite black (smoke brown is perhaps the proper term). The growth on potato was not wrinkled. The substratum and the fluid in the bottom of the tube also became brown. The rapidity and the degree of pigmentation seem to depend on the slightly varying composition of the potato, apparently on the amount of glucose present. No gas was formed in any of the many potato cultures. No acid was detected in any stage of the growth of the cultures, not even when tested at the end of the first twenty-four hours. The potato cultures, which were slightly acid on the start (normal acidity of the tuber), soon became strongly alkaline to litmus paper. With Nessler's solution the alkaline potato cultures gave an immediate, copious, orange-yellow reaction, indicating ammonia. These cultures developed a peculiar odor, often noticed in rotting potatoes, but not specially disagreeable. This odor was likened by one person to the smell of sour bran. Its chemical nature has not been determined. The cylinders did not fall into pieces, but retained their shape for several weeks.

"*Gas Production.*—No gas appeared in any of the many cultures. The organism is not a gas producer.

"*Relation to Oxygen.*—This bacillus appears to be strictly aërobic. If ever facultative anaërobic, it is not so with any of the carbohydrates yet tested.

"*Acids*—No acid reaction could be detected in any stage of any of the cultures. Potato cultures only twenty-four hours old and which were acid on the start (normal acidity of the tuber) gave a decided alkaline reaction to litmus paper. If any acid whatever is formed it is masked by the presence of alkali and is not butyric acid.

"*Alkalies.*—This organism is a very vigorous alkali producer. On warming the cultures over a gas flame or on placing the blued strips of litmus paper on a warm glass plate the alkaline reaction quickly disappears. On adding a few drops of Nessler's reagent, as already stated, a copious orange-yellow precipitate is at once developed. This would indicate that at least a part of the alkali is due to ammonia. Probably amine bases are also present.

"The bacillus grows well in the thermostat at 37° C.—possibly a trifle better than outside at summer temperatures ranging from 25° to 32° C. Under either condition it grows rapidly. It still grew readily from bouillon cultures after several weeks' exposure to 37 C. (three weeks' exposure in one case, seven weeks' exposure in another).

"*Pigments.*—A brown pigment is formed in course of a few days in the host plants (potato, tomato, etc.), and in culture media containing grape, fruit, or cane sugar (nutrient agar, steamed potato, fermentation tubes). This pigment is soluble in water and glycerin. It is insoluble in ethyl alcohol, ether, chloroform, xylol, and carbon bisulphide."

"BACILLUS HYACINTHI (Wakker).

"*Pseudomonas hyacinthi* (Wakker).—A yellow, rod-shaped organism, multiplying by fission; ends rounded; single, in pairs, or fours, more rarely in the form of chains or filaments; motile by means of one polar flagellum. In the host plant, when the bundles are crowded full of the yellow slime and broken down, it is generally 0.8 to 1.2 by 0.4 to 0.6 μ. In alkaline beef broth or on agar it usually measures 1 to 2 by 0.4 to 0.6 μ. In old cultures rich in sugar it often grows out into long, slender chains, or into filaments (50 to 100 μ long) in which there are no distinct septa. Non-sporiferous. Color distinctly yellow, but somewhat variable. Chrome yellow to pale cadmium in the host plant, *i.e.*, bright yellow (Ridgway's nomenclature of colors). On culture media, when not interfered with by the brown pigment, generally gamboge, chrome yellow, or canary yellow, but sometimes paler. Old cultures on some media darken from the production of a soluble, pale-brown pigment. This feeble brown stain is best developed in hyacinth broth, in potato broth with peptone, on turnips, on radishes, and on banana rinds. It was not observed in acid or alkaline beef broth, on cocoanut flesh, on sugar beets, in nutrient starch jelly, in agar, or in gelatin, with or without sugar. This organism grows readily on potato cylinders standing in distilled water, but it never becomes copious or fills the water with a solid yellow slime, owing to its feeble diastatic action. Potatoes on which it has grown, even for several months, always give a strong starch reaction with iodine. It behaves the same on nutrient starch jelly free from assimilable sugars. It liquefies nutrient gelatin and Löffler's blood serum, but does so slowly, and will not liquefy gelatin at all if ten per cent cane sugar is added. Growth on nutrient agar or nutrient starch jelly is inhibited (unless the inoculation be from a solid culture and very copious) by the addition of ten per cent glycerol, and is greatly retarded by five-per-cent glycerol; even two and a half per cent of glycerol retarded growth. Growth in beef broth was much retarded by the addition of 1.5-per-cent sodium chloride. Organisms extremely sensitive to plant acids, including those of the hyacinth. Aërobic; doubtfully, if ever, facultative anaërobic; not a gas producer. Does not redden litmus milk, but makes it bluer, and slowly separates the casein from the whey by means of a lab ferment. Produces under some circumstances, and slowly, a small amount of non-volatile acid (slime acid?) with various sugars (grape, cane, etc.), which acid is frequently obscured by the moderate production of alkali. In the presence of air produces an organic acid (probably acetic) from ethyl alcohol dissolved in milk or bouillon. Inverts cane sugar, but apparently without the intervention of any enzyme. Will not grow on thirty-per-cent grape-sugar agar. Resists dry air very well, *i.e.*, more than forty-eight days when spread on cover glasses in thin layers.

"In Dunham's solution with methylene blue the color is reduced in a few days, but re-oxidizes quickly on shaking; final color (fifty-six days) bright blue. In Dunham's solution with indigo carmine the color changes to a bright blue, which persists for a long time; final color yellowish. In Dunham's solution with rosolic acid and enough HCl to render the fluid yellowish, *Ps. hyacinthi* did not redden the fluid, but made it colorless, the bacterial precipitate becoming rosy or salmon-colored. Produces indol slowly in peptonized beef broth and in peptonized Uschinsky's solution; does not produce nitrites in these solutions. Does not reduce potassium nitrate to nitrite in peptonized beef bouillon. Not a strong-smelling germ. Not readily destroyed by its own decomposition products except in media containing alcohol.

"Will not grow in the thermostat at 37° C., and grows very feebly on some media and not at all on others at 34° to 35° C. Optimum temperature 28° to 30° C., or thereabouts. Minimum temperature approximately 4° C. Thermal death point (ten minutes' exposure), 47.50° C.; nearly all the rods are killed at 47° and a great many at 46.50° C. Did not grow at room temperature after six days' exposure in alkaline beef broth in the thermostat at 35° to 36.35°. Does not grow well in Uschinsky's solution. Grows much better in Uschinsky's solution when peptone is added to it. Grows well with a bright yellow color on cylinders of steamed cocoanut flesh, standing with one end in distilled water.

"Pathogenic to hyacinths. Enters the plant through wounds, through the blossoms, etc., and multiplies in the vascular system, filling the vessels, especially those of the bulb, with a bright yellow slime consisting of bacteria. The walls of the vessels are destroyed and extensive cavities are formed in the bundles. The parenchyma around the bundles is also involved, but only very slowly, the organism being a feeble destroyer of cell walls. The host plant is not rapidly destroyed, a year or more being necessary. The cells are first separated by solution of the middle lamella, but the wall itself seems finally to disappear. The cavities contain innumerable bacteria mingled with fragments of the dissolved bundles and of the surrounding parenchyma.

"First described by Dr. J. H. Wakker from the Netherlands, where it often causes serious losses in the hyacinth gardens. Not known to occur in any other part of the world" (E. F. Smith).

BACILLUS CAMPESTRIS (Pammel).

The cause of brown rot in Cruciferous plants.

"*Pseudomonas campestris* (Pammel).—Yellow, rod-shaped, motile micro-organism. Size and color varying according to substratum, food supply, etc. Generally 0.7 to 3.0 by 0.4 to 0.5 μ. Color dull wax yellow or canary yellow. Occasionally as bright as light cadmium or as pale as primrose yellow (Ridgway's color scale). One polar flagellum. Non-sporiferous, so far as known. Pathogenic for various Cruciferous plants, entering and dwarfing or destroying the host plant through the vascular system, which becomes decidedly brown. Aërobic but, so far as known, not a gas or acid producer, *i.e.*, not facultative anaërobic. Forms cavities around the bundles but seems to be only feebly destructive to cellulose. Produces a brown pigment in the host plant and on steamed Cruciferous substrata, especially the turnip. Grows very rapidly on steamed potato cylinders at room temperatures, but without odor or the formation of any brown pigment. Liquefies gelatin. Grows feebly at 7° C., better at 10° C., but still feebly; grows well at 17° to 19° C.; grows luxuriantly at 21° to 26° C.; grows very feebly at 37° to 38° C.; will not grow at 40° C.; and is killed by ten minutes' exposure to 51° C. Organism closely related to Wakker's Bacterium hyacinthi, from which it differs, so far as I have been able to observe, chiefly in its pathogenic properties, its duller yellow color and its higher thermal death point" (E. F. Smith).

PSEUDOMONAS STEWARTI (Smith).

"A medium-sized rod rounded at the end and motile by means of one polar flagellum, size 0.5 to 0.9 by 1 to 2 μ, no spores observed; found in enormous numbers in the vascular bundles of corn (*Zea mays*) associated with a destructive disease of which it is probably the cause; color in the host plant and in culture media yellow (buff to chrome or ochre, occasionally a pale, dirty yellow); aërobic and facultative anaërobic; grows in all ordinary culture media; bears alkali well (soda) and plant acids extremely well; grows luxuriantly in Uschinsky's solution; growth enormously stimulated by cane sugar, grape sugar, and galactose; growth not favored by five-per-cent doses of lactose, maltose, dextrin, mannite, or glycerin in nutrient starch jelly; diastatic action feeble, *i.e.*, able to obtain food from starch only with much difficulty; produces alkalies in all sorts of media and acids in the presence of grape and cane sugar; reduces litmus slowly; does not liquefy gelatin (Stewart); does not liquefy Löffler's blood serum; grows well at summer temperatures of 25° to 30° C.; does not die out quickly in culture media; does not produce gas; sensitive to light (Stewart); occurs in New York and Michigan and may be looked for in all parts of the United States" (E. F. Smith).

BACILLUS AMYLOVORUS (Burrill).

Described by Burrill (1880) as the cause of pear blight. Etiological relation to this disease confirmed by Arthur (1884 to 1887) and by Waite (1891 to 1895).

"Beginning in the spring the germs on the new growth of the season first appear on the negative discs of the blossoms. The bacilli live and multiply in the nectar and are able to enter the nectar glands without a puncture or injury, and thus normally get inside their hosts. The distribution from flower to flower and tree to tree is through the agency of insects, mainly flower-visiting source. Infection also occurs on the young shoots and less frequently on the fleshy bark through injuries. Insects and birds are agents of distribution and inoculation in these cases. No evidence could be found that the germs are carried by the wind. The blight germs usually die out in the twigs which are blighted and dead, but in certain cases the germs manage to keep alive through the summer by making slow progress in the fleshy bark. Such cases may succeed in living over winter. Winter weather is favorable to the longevity on account of the moisture and low temperature. The cases of "hold over" blights start off again in spring and exude quantities of gummy matter full of the bacilli. This is visited by insects, especially flies and wasps, and carried on to the newly opened flowers, thus completing the life cycle.

"An oval rod-like bacillus 0.6–0.8 μ by 1 to 6 μ long. Constant in diameter but varying greatly in length. Occurs singly or in young cultures in pairs, chains, or masses. Stains readily with the ordinary aniline dies either watery or alcoholic solutions. Has no capsule, but is supplied with several flagella scattered over the surface. It is actively motile. Does not produce spores. On nutrient beef and potato broth produces first a strong turbidity and a slight granular pellicle on the surface, which breaks up and settles to the bottom. The color of the mass is milky white on all solid media.

"On agar plates the outside colonies at ordinary temperature (18° to 20° C.) reach a diameter of about one millimetre in forty-eight hours, and at the end of a week become five to six millimetres across. A temperature of 36° to 37° C. starts the growth more promptly, but results in a feebler ultimate development.

"The addition of malic or citric acid in small amounts so as to acidify the agar, increases the vigor of growth, while an excess of alkali diminishes it. On gelatin made from the commercial brands the opposite effect is produced. Gelatin should be neutral to phenolphthalein to insure vigorous development. There is moderate liquefaction in good gelatin culture. A moderate growth is made on sterile potato cylinders.

"In the fermentation tube it decomposes sugar without the formation of gas. It is most vigorous on maltose, the cultures becoming strongly acid, and is slightly less so on cane sugar, dextrose, and levulose. It is aërobic and facultative anaërobic. It produces no pigment or coloring matter of any sort, and no odor. It does not decompose starch. Its principal food consists of nitrogenous matter, sugars, and probably, to some extent, certain organic acids, the very substances which occur in vigorous, young, growing tissues of the host. Certain statements formerly made are now known to be erroneous.

"The germ mass is said to be yellowish-white on potato. This could only come from an impure culture, as the true pear-blight germ is always white. Gas, in some places CO_2, is said to be formed. This never occurs. Butyric acid is said to be one of the products of its decomposition. The germ produces acid but never butyric. Starch is said to be decomposed and used as a food, but so far we have never been able to demonstrate this. The germ is said to live over winter in the soil. In our search we have failed to find it in such places, and its life cycle is complete without it" (Waite).

BACILLUS TRACHEIPHILUS (Smith).

The cause of "wilt" in various species of *Cucurbitaceæ*—cucumbers and melons.

"Bacilli, often two or three times as long as broad, of medium size; solitary or in pairs, occasionally in chains of four. The dimensions vary greatly in the infected plant; many rods are 1.2 to 2.5 μ long by 0.5 to 0.7 μ broad

In cultures the dimensions vary still more. In recent cultures the bacilli exhibit active movements, which are soon lost. The bacilli are often associated in viscous masses, forming milk-white drops, which when touched with a platinum needle may be drawn out into long threads. This viscosity appears to be due to a swollen and partially liquefied capsule, which may be demonstrated under the microscope in stained or unstained preparations. Does not form spores. Grows in bouillon, Dunham's solution, etc. Does not form a surface film or a deposit at the bottom of the test tube, but the culture medium is slightly clouded. Grows very slowly or not at all in gelatin and does not liquefy. Upon agar-agar it grows as a thin, smooth, milk-white, sticky layer, which extends only a short distance from the point of inoculation. In stab cultures it grows all along the line of puncture, forming, after a time, finger-like projections, which under a lens are seen to be finely granular. Upon potato it forms a thin, smooth, white, moist-looking layer, which only extends a short distance from the line of inoculation. The color of the growth resembles that of the potato, and is much whiter than that of most bacteria. It produces no pigment and causes no change in the color of the potato. In culture solutions containing dextrose, saccharose, lactose, or maltose no gas is developed. It does not cause coagulation of milk. It grows best in alkaline media. It is destroyed by a temperature of 43° C. maintained for ten minutes. Cultures in liquid media or on potato usually die out within three weeks. It stains best with carbol-fuchsin solution. In properly stained preparations it is seen to have a capsule and flagella—in some bacilli one flagellum at each extremity of the rod, while in others there are more". (Smith).

XVI.

PATHOGENIC ANAËROBIC BACILLI.

STRICTLY anaërobic bacilli are not able to multiply in the blood of living animals ; but some of them may multiply in the subcutaneous connective tissue or in the muscles, when introduced by inoculation, and are pathogenic because of the local inflammatory or necrotic processes to which they give rise, or because they produce soluble toxic substances which are absorbed and cause death by their special action upon the nervous system or by general toxæmia.

BACILLUS TETANI.

Synonyms.—The bacillus of tetanus ; Tetanusbacillus, *Ger.*

Nicolaier (1884) produced tetanus in mice and rabbits by introducing garden earth beneath their skin, and showed that the disease might be transmitted to other animals by inoculations with pus or cultures in blood serum containing the tetanus bacillus, which, however, he did not succeed in obtaining in pure cultures. Carle and Rattone (1884) showed that tetanus is an infectious disease, which may be transmitted by inoculation from man to lower animals—a fact which has since been verified by the experiments of Rosenbach and others. Obtained in pure cultures by Kitasato (1889).

The writer produced tetanus in a rabbit in 1880 by injecting beneath its skin a little mud from the street gutters in New Orleans. The tetanus bacillus appears to be a widely distributed microörganism in the superficial layers of the soil in temperate and especially in tropical regions. In Nicolaier's experiments it was not found in soil from forests or in the deeper layers of garden earth.

Morphology.—Slender, straight bacilli, with rounded ends, which may grow out into long filaments. Spores are developed at one extremity of the bacilli, which are spherical in form and considerably greater in diameter than the rods themselves, giving the spore-bearing bacilli the shape of a pin.

Stains with the usual aniline colors and also by Gram's method. The method of Ziehl may be employed for double-staining bacilli and spores.

Biological Characters.—An *anaërobic, liquefying, motile* bacillus. *Forms spores.* Grows at the room temperature, in the absence of oxygen, in the usual culture media. Grows best at a temperature of 36° to 38° C.; in nutrient gelatin, at 20° to 25° C., development is first seen at the end of three or four days ; does not grow at a temperature below 14° C. Spores are formed in cultures kept in the incubating oven at 36° C., at the end of thirty hours ; in gelatin cultures at 20° to 25° C., at the end of a week (Kitasato). The bacilli exhibit voluntary movements which are not very active ; those containing spores are not motile. It may be cultivated in an atmosphere of hydrogen, but does not grow in the presence of oxygen—strictly anaërobic—or in an atmosphere of carbon dioxide. The addition of one and one-half to two per cent of grape sugar to nutrient agar or gelatin causes the development to be more rapid and abundant. The culture medium should have a feebly alkaline reaction.

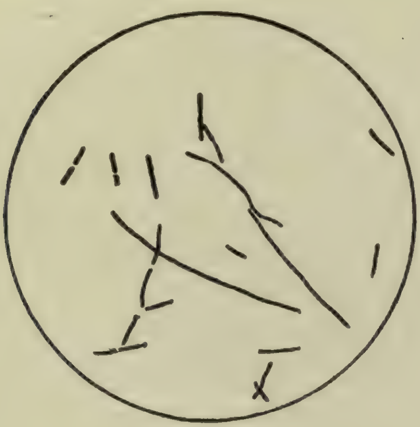


FIG. 159.

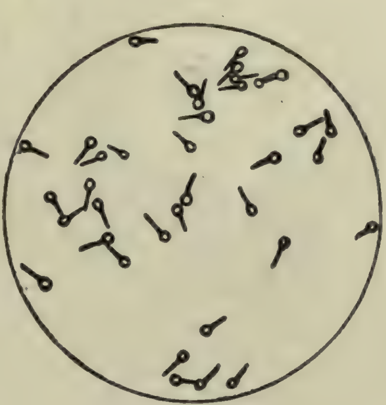


FIG. 160.

FIG. 159.—Tetanus bacillus, from a gelatin culture. × 1,000. From a photomicrograph by Pfeiffer.

FIG. 160.—Tetanusbacillus, from an agar culture ; spore-bearing rods. × 1,000. From a photomicrograph by Pfeiffer.

Colonies in *gelatin plates,* in an atmosphere of hydrogen, resemble somewhat colonies of Bacillus subtilis, the opaque central portion being surrounded by a circle of diverging rays ; liquefaction is, however, much slower, and the resemblance is lost after a short time. Older colonies resemble the colonies of certain microscopic fungi, being made up of diverging rays. In long *gelatin stab cultures* development occurs along the line of puncture, at a considerable distance below the surface, in the form of a radiate outgrowth ; the gelatin is slowly liquefied, and a small amount of gas is at the same time formed. In peptonized bouillon having a slightly alkaline reaction, under hydrogen gas, the development is abundant and the cultures give off a characteristic odor—"brenzlichen Geruch" (Kitasato).

According to Kitasato, *blood serum* is not a very favorable medium for the growth of the tetanus bacillus, and—contrary to the statement of Kitt, Tizzoni, and others—it does not cause liquefaction of this medium.

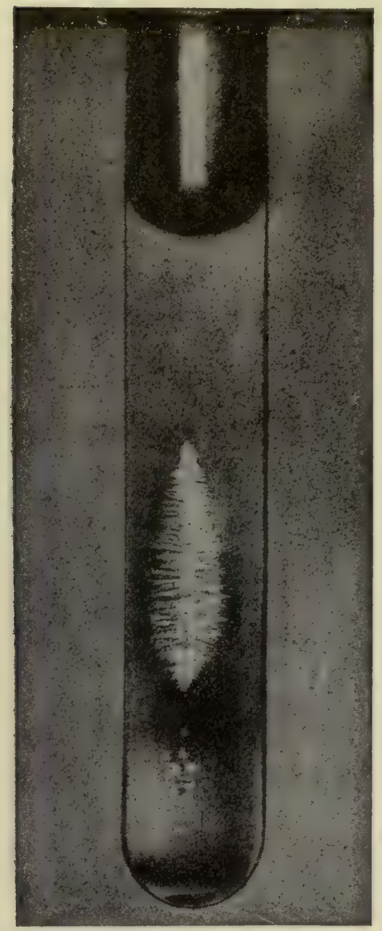


FIG. 161.—Culture of Bacillus tetani in nutrient gelatin. (Kitasato.)

The spores of the tetanus bacillus retain their vitality for months in a desiccated condition, and are not destroyed in two and one-half months when present in putrefying material (Turco). They withstand a temperature of 80° C. maintained for an hour, but are killed by five minutes' exposure to steam at 100° C. They are not destroyed in ten hours by a five-per-cent solution of carbolic acid, but did not grow after fifteen hours' exposure in the same solution. A five-per-cent solution of carbolic acid, to which 0.5 per cent of hydrochloric acid has been added, destroys them in two hours; in sublimate solution containing 1 : 1,000 of mercuric chloride they are destroyed at the end of three hours, or in thirty minutes when 0.5 per cent of hydrochloric acid is added to the solution. Kitasato succeeded in obtaining pure cultures from the pus formed in the vicinity of inoculation wounds, by destroying the associated bacilli after the tetanus bacilli had formed spores. This was effected by heating cultures from this source for about an hour at a temperature of 80° C. The spores of the tetanus bacillus survived this exposure, and colonies were obtained from them in flat flasks especially devised for anaërobic cultures; from these colonies pure cultures in nutrient agar or gelatin—long stick cultures—or in peptonized bouillon were easily obtained.

BACILLUS ŒDEMATIS MALIGNI.

Synonyms.—Bacillus of malignant œdema; Vibrion septique (Pasteur).

Discovered by Pasteur (1877); carefully studied by Koch (1881).

This bacillus is widely distributed, being found in the superficial layers of the soil, in dust, in putrefying substances, in the blood of animals which have been suffocated (by invasion from the intestine), in foul water, etc.

It may usually be obtained by introducing beneath the skin of a rabbit or a guinea-pig a small quantity of garden earth. The animal dies within a day or two, and this bacillus is found in the bloody serum effused in the subcutaneous connective tissue for a considerable distance about the point of inoculation.

Morphology.—Bacilli from 3 to 3.5 μ long and 1 to 1.1 μ broad; frequently united in pairs, or chains of three elements; may grow out into long filaments 15 to 40 μ long—these are straight, or bent at an angle, or more or less curved. They resemble the bacillus of anthrax, but are not quite as broad, have rounded ends, and in stained preparations the long filaments are not segmented as is the case with the anthrax bacillus. By Löffler's method of staining they are seen to have flagella arranged around the periphery of the cells. Large, oval spores may be developed in the bacilli (not in the long filaments), which are of greater diameter than the rods, and produce a terminal or central swelling of the same, according to the location of the spore.

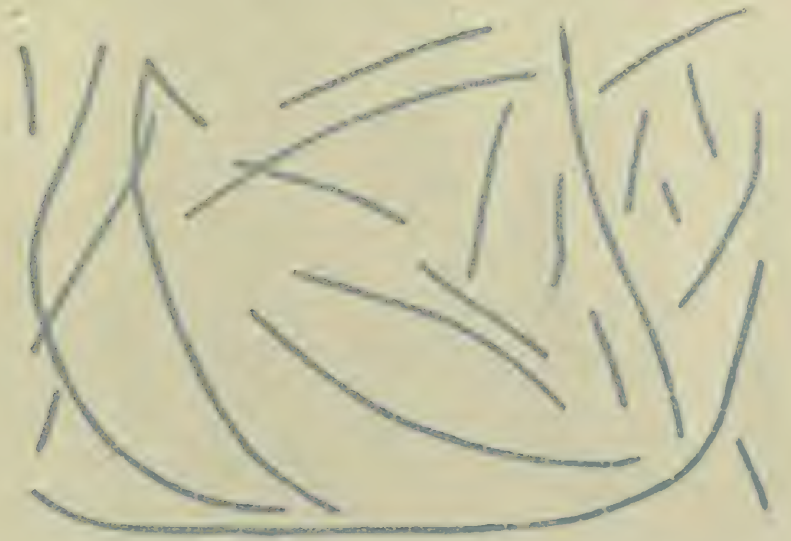


FIG. 162.—Bacillus œdematis maligni, from subcutaneous connective tissue of inoculated guinea-pig. × 950. (Baumgarten.)

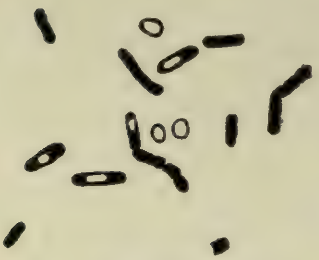


FIG. 163.—Bacillus œdematis maligni, from an agar culture, showing spores. × 1,000 From a photomicrograph. (Fränkel and Pfeiffer.)

Stains readily by the aniline colors usually employed, but is decolorized when treated by Gram's method.

In stained preparations the long filaments may present a somewhat granular appearance from unequal action of the staining agent.

Biological Characters.—A strictly *anaërobic*, *liquefying*, *motile* bacillus. *Forms spores.* Grows in the usual culture media when oxygen is excluded—in an atmosphere of hydrogen. Grows at the room temperature—better in the incubating oven at 37° C. The spores are formed most abundantly in cultures kept in the incubating oven, but may also be formed at a temperature of 20° C. In the bodies of animals which succumb to an experimental inoculation no spores are found immediately after death, but the bacilli multiply rapidly in the cadaver, and form spores when the temperature is favorable.

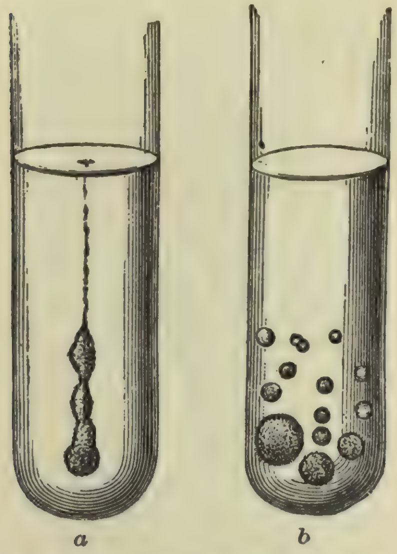


Fig. 164.—Bacillus œdematis maligni, cultures in nutrient gelatin; *a*, long stab culture: *b*, colonies at bottom of gelatin tube. (Flügge.)

The malignant-œdema bacillus may be cultivated in ordinary *nutrient gelatin*, but its development is more abundant when one to two per cent of grape sugar has been added to the culture medium. In deep stab cultures in this medium development occurs at first only near the bottom of the line of puncture; the gelatin is liquefied and has a grayish-white, clouded appearance; an abundant development of gas occurs, and as this accumulates the growth and liquefaction of the gelatin extend upward. A very characteristic appearance is obtained when the bacilli are mixed in a test tube with gelatin which has been liquefied by heat, and which is then allowed to solidify. Spherical colonies are developed, in the course of two or three days, in the lower portion of the gelatin; these are filled with liquefied gelatin of a grayish-white color, and when examined with a low power are seen to be permeated with a network of filaments, while the periphery presents a radiate appearance. In *nutrient agar* growth also occurs at the bottom of a deep puncture; it has an irregular, jagged outline and a granular appearance; the considerable development at the deepest portion and gradual thinning out above give the growth a club shape; in the incubating oven there is an abundant development of gas, which often splits up the agar medium and forces the upper portion against the cotton stopper. An abundant development of gas also occurs in cultures in *blood serum*, and the medium is rapidly liquefied; at a temperature of 37° it is changed in a few days to a yellowish fluid, at the bottom of which some irregular, corroded fragments of the solidified serum may be seen. In *agar plates*, placed in a close receptacle from which oxygen is excluded, cloudy, dull-white colonies are formed which have irregular outlines and under the microscope are seen to be made up of branching and interlaced filaments radiating from the centre. Cultures of the malignant-œdema bacillus give off a peculiar, disagreeable odor, which cannot, however, be designated as "putrefactive."

Pathogenesis.—Pathogenic for mice, guinea-pigs, rabbits, and, according to Kitt, for horses, dogs, goats, sheep, calves, pigs, chickens, and pigeons. According to Arloing and to Chauveau, cattle are immune. The disease is rarely developed except as a result of experimental inoculations, but horses occasionally have malignant œdema from accidental inoculation, and cases have been reported in man—"gangrène gazeuse." A small quantity of a pure culture injected beneath the skin of a susceptible animal gives rise to an extensive inflammatory œdema of the subcutaneous connective tissue and of the superficial muscles, which extends from the point of inoculation, especially towards the more dependent portions of the body. The bloody serum effused is without odor and contains little if any gas. But when malignant œdema results from the introduction of a little garden earth beneath the skin of a guinea-pig or other susceptible animal, the effused serum is frothy and has a putrefactive odor, no doubt from the presence of associated bacteria. Injections into the circulation do not give rise to malignant œdema, unless at the same time some bacilli are thrown into the connective tissue. While small animals usually die from an experimental inoculation with a moderately small quantity of a pure culture, larger ones (dogs, sheep) frequently recover. At the autopsy, if made at once, the bacilli are found in great numbers in the effused serum, but not in blood from the heart or in preparations made from the parenchyma of the various organs; later they may be found in all parts of the body as a result of post-mortem multiplication. This applies to rabbits and to guinea-pigs, but not to mice; in these little animals the bacilli may find their way into the blood during the last hours of life, and their presence may be demonstrated in smear preparations of blood from the heart or from the parenchyma of the spleen or liver. In mice the spleen is considerably enlarged, dark in color, and softened; in rabbits and guinea-pigs less so. With this exception the internal organs present no very notable pathological changes.

Animals which recover from malignant œdema are said to be subsequently immune (Arloing and Chauveau). Roux and Chamberlain have shown that immunity may be induced in guinea-pigs by injecting filtered cultures of the malignant-œdema bacillus (about one hundred cubic centimetres of a bouillon culture in three doses) into the abdominal cavity; or, better still, by the injection of filtered serum from animals which have recently succumbed to an experimental inoculation (one cubic centimetre repeated daily for seven or eight days).

BACILLUS CADAVERIS.

Obtained by the writer (1889) from pieces of liver and kidney, from yellow-fever cadavers, which had been preserved for forty-eight hours in an antiseptic wrapping, at the summer temperature of Havana; also in two cases from pieces of yellow-fever liver immediately after the autopsy; also from liver preserved in an antiseptic wrapping from comparative autopsies made in Baltimore.

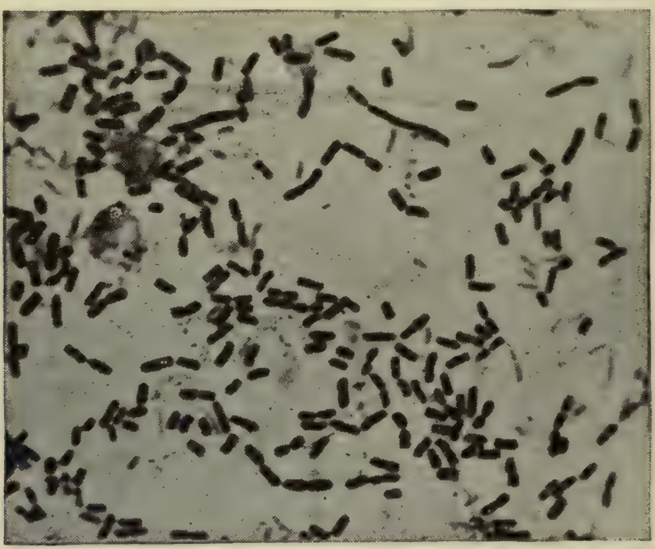


FIG. 165.—Bacillus cadaveris; smear preparation from liver of yellow-fever cadaver, kept twenty-four hours in an antiseptic wrapping. × 1,000. From a photomicrograph. (Sternberg.)

Morphology.—Large bacilli with square or slightly rounded corners, from 1.5 to 4 μ in length and about 1.2 μ broad; frequently associated in pairs; may grow out into straight or slightly curved filaments of from 5 to 15 μ in length.

Biological Characters.—An *anaërobic*, *non-motile* bacillus; not cultivated in nutrient gelatin; not observed to form spores.

Bacillus cadaveris is a strict anaërobic and is difficult to cultivate. I have succeeded best with nutrient agar containing five per cent of glycerin, removing the oxygen thoroughly by passing a stream of hydrogen through the liquefied medium. The colonies in a glycerin-agar roll tube (containing hydrogen and hermetically sealed) are opaque, irregular in outline, granular, and of

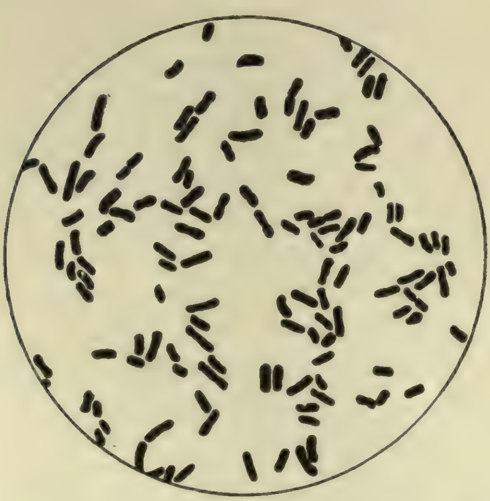


FIG. 166.—Bacillus cadaveris, from an anaërobic culture in glycerin-agar. × 1,000. From a photomicrograph. (Sternberg.)

a white color by reflected light. The culture medium acquires an acid reaction as a result of the development of the bacillus.

Liver tissue containing this bacillus, after having been kept in an antiseptic wrapping for forty-eight hours, has a fresh appearance, a very acid reaction, and is without any putrefactive odor.

Pathogenesis.—Liver tissue containing this bacillus is very pathogenic for guinea-pigs when injected subcutaneously, and causes an extensive inflammatory œdema extending from the point of inoculation. Pure cultures of the bacillus are less pathogenic, and the few experiments which I made in Havana gave a somewhat contradictory result, recovery having occurred in one guinea-pig which received a subcutaneous injection of ten minims of liquid from an anaërobic culture in glycerin-agar, while another died at the end of twenty hours from a subcutaneous injection of three minims, with extensive inflammatory œdema in the vicinity of the point of inoculation.

BACILLUS OF SYMPTOMATIC ANTHRAX.

Synonyms.—Rauschbrandbacillus, *Ger.*; Bacille du charbon symptomatique, *Fr.*

First described by Bollinger and Feser (1878); carefully studied and its principal characters determined by Arloing, Cornevin, and Thomas (1880–83).

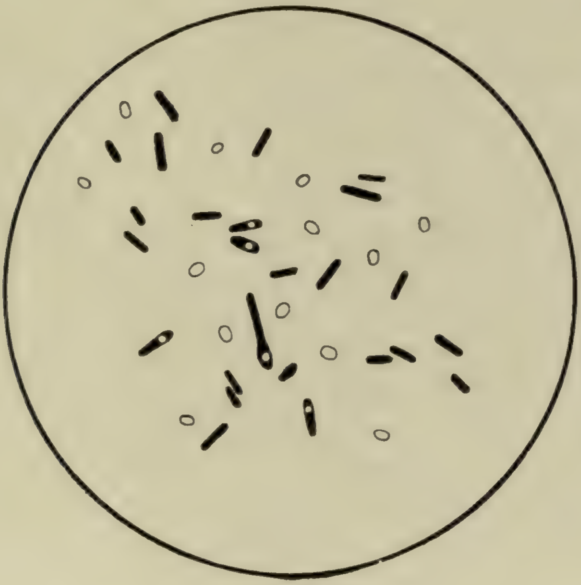


FIG. 167.

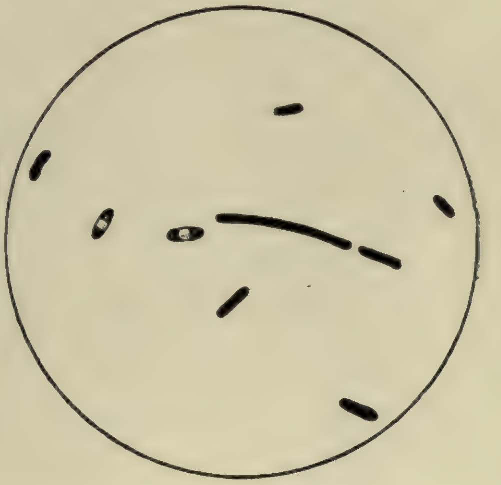


FIG. 168.

FIG. 167.—Bacillus of symptomatic anthrax, from an agar culture. × 1,000. From a photomicrograph. (Fränkel and Pfeiffer.)

FIG. 168.—Bacillus of symptomatic anthrax, from muscles of inoculated guinea-pig. From a photomicrograph. (Roux.)

Found in the affected tissues of animals—principally cattle—suffering from "black leg," "quarter evil," or symptomatic anthrax (Fr., "charbon symptomatique"; Ger., "Rauschbrand"). The disease prevails during the summer months in various parts of Europe, and is characterized by the appearance of irregular, emphysematous swellings of the subcutaneous tissue and muscles, especially over the quarters, hence the name "quarter evil." The muscles in the affected areas have a dark color and contain a bloody serum in which the bacillus is found.

Morphology.—Bacilli with rounded ends, from three to five μ long and 0.5 to 0.6 μ broad; sometimes united in pairs, but do not grow out into filaments. The spores are oval, somewhat flattened on one side, thicker than the bacilli, and lie near the middle of the rods, but a little nearer to one extremity. The bacilli containing spores are somewhat spindle-formed (Kitasato). "Involution forms" are quite common in old cultures or in unfavorable media; in such cultures variously distorted and often greatly enlarged bacilli may be seen, some being greatly swollen in the middle — spindle-shaped. When properly stained, by Löffler's method, a number of flagella are seen around the periphery of the cells.

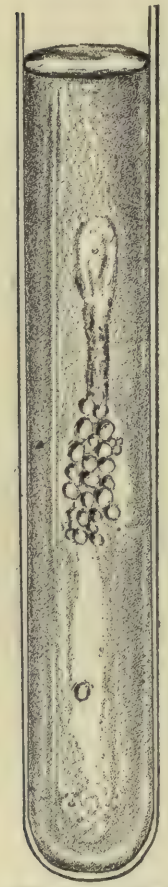


Fig. 169. — Bacillus of symptomatic anthrax; long stab culture in nutrient gelatin, ten days at 18°–20° C. (Kitasato.)

Stains with the aniline colors usually employed, but not by Gram's method. Spore-bearing bacilli may be double-stained by first staining the spores by Ziehl's method, and then the bacilli with a solution of methylene blue.

Biological Characters.—An *anaërobic, liquefying, motile* bacillus. *Forms spores.* Grows at the room temperature in the usual culture media, in the absence of oxygen, in an atmosphere of hydrogen, but not in carbon dioxide. This bacillus grows more rapidly and abundantly in nutrient agar or gelatin to which 1.5 to 2 per cent of grape sugar or five per cent of glycerin has been added. Colonies in gelatin, in an atmosphere of hydrogen, are at first spherical, with irregular outlines and a wart-like surface; later the gelatin is liquefied around them, and radiating filaments grow out into the gelatin, so that by transmitted light they present the appearance of an opaque central mass with an irregular surface surrounded by rays. In stab cultures in *nutrient* gelatin, at 20° to 25° C., at the end of two or three days development occurs at the bottom of the line of puncture to within about two fingers' breadth of the surface; the gelatin is slowly liquefied and considerable gas is formed. In old cultures the growth and liquefaction of the gelatin extend nearly to the surface. In *agar* stab cultures, in the incubating oven, development begins within a day or two and extends to within one finger's breadth of the surface; considerable gas is evolved, and the cultures have a peculiar, acid, penetrating odor. Development is most rapid at 36° to 38° C., but may occur at a temperature of 16° to 18° C.—not lower than 14°. Spores are quickly formed in cultures kept in the incubating oven—not so quickly at the room temperature. These withstand a temperature of 80° C. maintained for an hour, but are killed in five minutes by a temperature of 100° C. (in steam). In the bodies of infected animals spores are not formed until after the death of the animal, at the end of twenty-four to forty-eight hours (Kitasato).

The spores are destroyed by a five-per-cent solution of carbolic acid in ten hours, and the bacilli, in the absence of spores, in five minutes ; a 1 : 1,000 solution of mercuric chloride destroys the spores in two hours (Kitasato). According to Kitasato, certain shining bodies of irregular form, which stain readily with the aniline colors, are to be seen in the rods as they are found in the bloody serum from an animal recently dead ; but these are not spores, as some bacteriologists have supposed.

Pathogenesis.—Cattle, which are immune against malignant œdema, are most subject to infection by the bacillus of symptomatic anthrax, and the disease produced by this anaërobic bacillus prevails almost entirely among them ; horses are not attacked spontaneously —*i.e.*, by accidental infection—and when inoculated with a culture of this bacillus present only a limited local reaction. Swine, dogs, rabbits, fowls, and pigeons have but slight susceptibility, but the researches of Arloing, Cornevin, and Thomas, and of Roger show that by the addition of a twenty-per-cent solution of lactic acid to a culture its virulence is greatly increased, and animals which have but little susceptibility, like the rabbit or the mouse, succumb to such injections ; similar results were obtained by Roger by the simultaneous injection of sterilized or non-sterilized cultures of Bacillus prodigiosus or of Proteus vulgaris.

Klein (1894) has obtained from the spleen of sheep a bacillus which corresponds with the bacillus of malignant œdema in every respect, except that it proved to be without pathogenic power—"a non-virulent variety of the Rauschbrand bacillus" (Klein).

BACILLUS ŒDEMATIS MALIGNI NO. II (Novy).

Obtained by Novy (1894) from the subcutaneous œdema in guinea-pigs which were inoculated with a solution of milk-nuclein, which had been prepared from fresh casein.

Morphology.—Bacilli with rounded ends, usually solitary, from 2.5 to

5 μ long and from 0.8 to 0.9 μ broad. Occasionally short and straight filaments, 8 to 14 μ long, are seen—very rarely these reach a length of 22 to 35 μ. Long and slender spiral filaments are found in pure cultures which are believed to be gigantic flagella. These are seen in preparations stained with gentian violet as unstained spiral filaments, usually from 17 to 25 μ long; some are of uniform thickness and others spindle-formed, having a thickness of 1.7 to 2.6 μ in the middle, and tapering to a scarcely visible line at the extremities. These flagella are readily stained by Löffler's method. They are attached to the periphery of the rods, as in the typhoid bacillus. In artificial cultures they are usually from 40 to 50 μ long. With reference to the peculiar spindle-formed bodies found in the cultures Novy says: "As to the character of these gigantic flagella little can be said. Löffler, who, so far as I know, was the first to observe these singular forms, regarded them as bundles or collections of flagella."

Although at first inclined to doubt this, Novy says, in a postscript to his paper, that an examination of photo-micrographs, which had been made to accompany it, convinces him that Löffler's explanation is probably correct.

Biological Characters.—An *anaërobic*, *motile* bacillus. The motions are not active, but consist in a very moderate to-and-fro swinging motion. Does not form spores. Does not grow at the room temperature. Grows at temperatures of 24° to 38° C. The best media for its development are slightly alkaline bouillon, gelatin, or agar, containing two per cent of glucose. May be cultivated in a vacuum or in an atmosphere of hydrogen, carbon dioxide, or illuminating gas. Also in long stick cultures in agar. In *glucose-agar plates* colonies develop in fifteen hours at 38° C. These appear as small, white masses the size of a pin's head, which, under the microscope, appear to be made up of thickly felted threads. The smaller colonies appear as a network of branching lines, very similar to the colonies of the tetanus bacillus; larger colonies have a dark centre, with an irregular, fringed margin, and are surrounded by delicate filaments. In *glucose-agar stab cultures* growth occurs along the line of puncture to within one cubic centimetre of the surface, but is not as abundant as the growth of the bacillus of malignant œdema or of symptomatic anthrax. At 38° C. development occurs within twelve to sixteen hours, and has reached its maximum at the end of twenty-four hours. An abundant development of gas occurs, which splits up the agar and forces the upper portion towards the top of the tube. The development of gas is most abundant in alkaline media, being almost absent in media having a neutral or acid reaction. The most favorable medium is a fresh alkaline bouillon containing two per cent of gelatin, of glucose, and of peptone.

Pathogenesis.—Pathogenic for rabbits, guinea-pigs, white mice, white rats, pigeons, and cats. Death usually results in from twelve to thirty-six hours after the subcutaneous injection of one-tenth to one-fourth cubic centimetre of a pure culture. At the autopsy an extensive subcutaneous œdema is found extending from the point of inoculation. The fluid in the brawny connective tissue is usually colorless, sometimes of a pale-red color. A small amount of gas is commonly present. The pleural cavities contain an enormous amount of serous exudate, which at first is fluid, but when the autopsy is delayed becomes gelatinous. In rabbits and guinea-pigs the amount of this serum obtained from the pleural cavities may be from fifty to sixty cubic centimetres. The bacilli are usually not very numerous in this serum from the subcutaneous tissues and pleural cavity.

Kerry (1894) has described a "new pathogenic anaërobic bacillus" which resembles that of Novy in several particulars. It does not grow at the room temperature, does not form spores, and is pathogenic for mice, rats, rabbits, and guinea-pigs; it forms "very long and thick flagella, which may be *spiralig gechlängelt.*" This bacillus was obtained from a guinea-pig inoculated with dried blood (suspended in water containing lactic acid and glucose) which had been obtained from a cow that was supposed to have died of Rauschbrand.

BACILLUS AËROGENES CAPSULATUS.

Found by Welch in the blood vessels of a patient with thoracic aneurism opening externally; autopsy made in cool weather eight hours after death—the vessels found full of gas bubbles.

Morphology.—Straight or slightly curved bacilli with slightly rounded or sometimes square-cut ends; a little thicker than Bacillus anthracis, and varying in length—average length 3 to 6 μ; long threads and chains are occasionally seen. The bacilli, both from cultures and in the animal body, are enclosed in a transparent capsule.

Biological Characters.—An *anaërobic*, *non-motile*, *non-liquefying* bacillus. Does not form spores. Grows in the usual culture media, in the absence of oxygen, at the room temperature, and produces an abundant development of gas in all. In *nutrient gelatin* there is no marked liquefaction, but the gelatin is slightly peptonized. In *agar*, colonies are developed which are usually one to two millimetres in diameter, but may attain a diameter of one centimetre; they are grayish-white in color and in the form of flattened spheres, ovals, or irregular masses, beset with little projections or hair-like processes. *Bouillon* is rendered diffusely cloudy, with an abundant white sediment. *Milk* is coagulated in one or two days. The cultures in agar and bouillon have a faint odor, comparable to that of stale glue. Upon *potato* a pale grayish-white layer is developed; growth occurs at 18° to 20° C., but is much more rapid at 30° to 37° C. Bouillon cultures are sterilized by exposure to a temperature of 58° C. for ten minutes.

Pathogenesis.—"Quantities up to 2.5 cubic centimetres of fresh bouillon cultures were injected into the circulation of rabbits without any apparent effect, except in one instance in which a pregnant rabbit was killed, by the injection of one cubic centimetre, in twenty-one hours. If the animal is killed shortly after the injection the bacilli develop rapidly after death, with an abundant formation of gas in the blood vessels and organs, especially the liver. At temperatures of 18° to 20° C. the vessels, organs, and serous cavities may be full of gas in eighteen to twenty-four hours, and at temperatures of 30° to 32° C. in four to six hours, when one cubic centimetre of a bouillon culture has been injected into the circulation shortly before death."

It is suggested by Welch and Nuttall that in some of the cases in which death has been attributed to the entrance of air into the veins, the gas found at the autopsy may not have been atmospheric air, but may have been produced by this or some similar microörganism entering the circulation and developing after death.

In a paper published in the Bulletin of the Johns Hopkins Hospital (September, 1900) Professor Welch says: "Our further studies of the gas bacillus obtained from different sources have shown a moderate range of variation in some of its properties. This is true especially of spore formation, rapidity of liquefaction of gelatin, presence of capsules, and virulence."

This bacillus has been shown by recent researches to be widely distributed in nature, its natural habitat being the intestinal canal of man and lower animals and the soil. It has considerable importance in human pathology, having been found in various localized infectious processes in the subcutaneous tissues, the uterus, the urinary tract, the liver, the lungs, and the pleural cavities.

XVII.

PATHOGENIC SPIRILLA.

SPIRILLUM OBERMEIERI.

Synonyms.—Spirochæte Obermeieri; Spirillum of relapsing fever; Die Recurrensspirochäte.

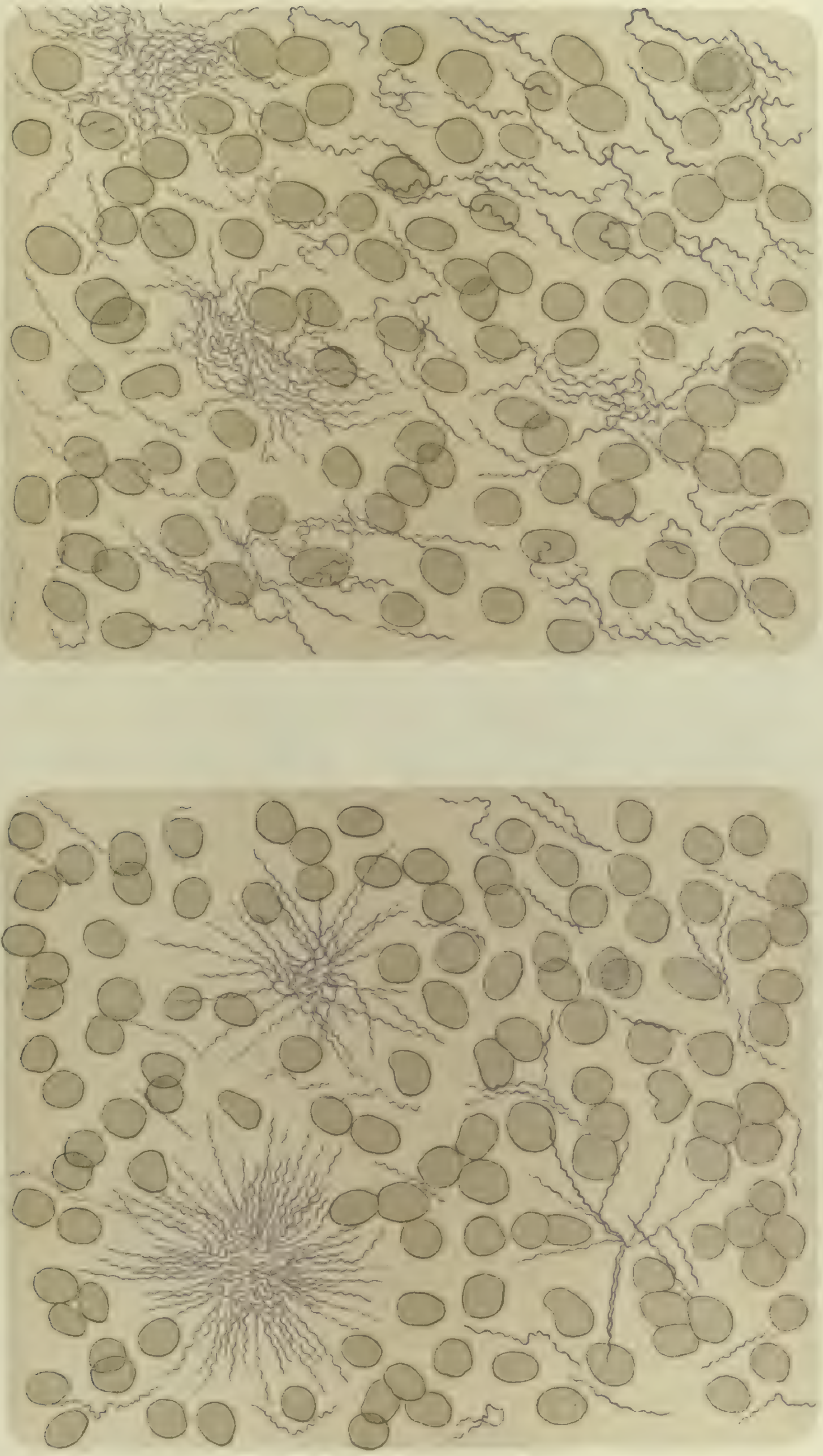
Discovered by Obermeier (1873) in the blood of persons suffering from relapsing fever.

This spirillum is present, in very great numbers, in the blood of relapsing-fever patients during the febrile paroxysms. It has not been found under any other circumstances, and its etiological relation to the disease with which it is associated is generally admitted.

Morphology.—Very slender, flexible, spiral or wavy filaments, with pointed ends; from sixteen to forty μ in length and considerably thinner than the cholera spirillum—about 0.1 μ. Koch has demonstrated the presence of flagella (Eisenberg).

Stains readily with the aniline colors, especially with fuchsin, Bismarck brown, and in Löffler's solution of methylene blue.

Biological Characters.—An *aërobic, motile* spirillum which has not been cultivated in artificial media. This spirillum appears to be a strict parasite, whose habitat is the blood of man. The disappearance of the parasite from the blood soon after the termination of a febrile paroxysm, and its reappearance during subsequent paroxysms, have led to the inference that it must form spores, but this has not been demonstrated. In fresh preparations from the blood the spirillum exhibits active progressive movements, accompanied by very rapid rotation in the long axis of the spiral filaments, or by undulatory movements. The movements are so vigorous that the comparatively large red blood corpuscles are seen, under the microscope, to be thrown about by the slender spiral filaments, which it is difficult to see in unstained preparations. When preserved in a one-half-per-cent salt solution they continue to exhibit active movements for a considerable time. Efforts to cultivate this spirillum in artificial media have thus far been unsuccessful, although Koch has observed an increase in the length of the spirilla and the formation of a tangled mass of filaments.



Spirillum Obermeieri in blood of two monkeys, inoculated after removal of spleen.
(Soudakewitch).

In experiments made by Heydenreich the spirillum was found to preserve its vitality (motility) for fourteen days at a temperature of 16° to 22° C., for twenty hours at 37°, and at 42.5° for two or three hours only.

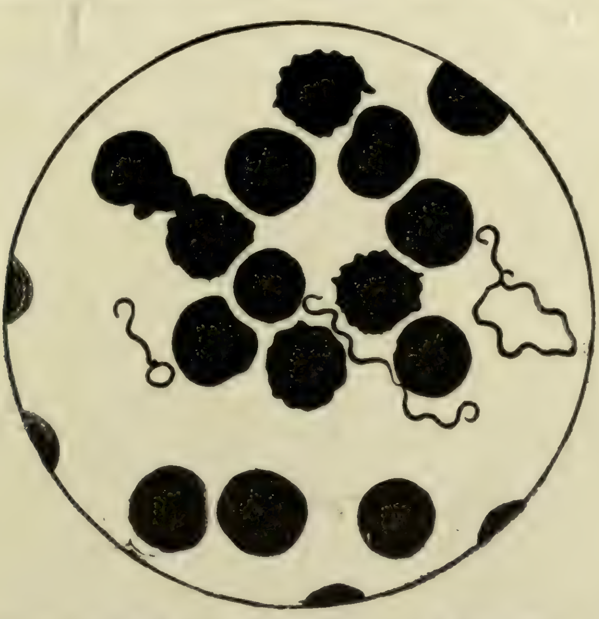


FIG. 170.—Spirillum Obermeieri in blood of man. × 1,000. From a photomicrograph. (Fränkel and Pfeiffer.)

Pathogenesis.—Causes in man the disease known as relapsing fever. Münch and Moczutkowsky have produced typical relapsing fever in healthy persons by inoculating them with blood containing the spirillum of Obermeier. The spirilla are found in the blood during the febrile paroxysm, and for a day or two, at the outside, after

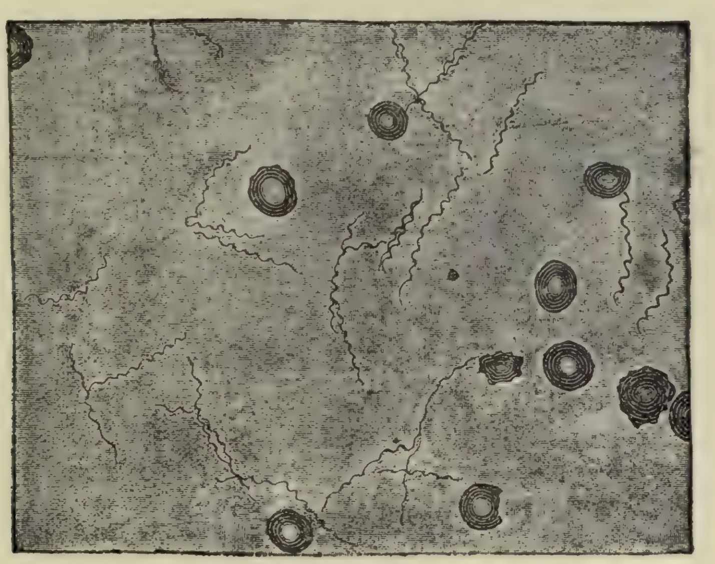


FIG. 171.—Spirillum Obermeieri in blood of an inoculated ape. × 700. (Koch.

its termination ; sometimes they are present in great numbers, and at others can only be found by searching several microscopic fields; they are not present in the various secretions—urine, sweat, saliva, etc. In fatal cases the principal pathological changes are found in the spleen, which is greatly enlarged, and in the liver and marrow of the bones, which contain inflammatory and necrotic foci. Koch and Carter have succeeded in transmitting the disease to monkeys by subcutaneous inoculations with small amounts of defibrinated blood containing the spirillum. After an incubation period of several days typical febrile paroxysms were developed, during which the actively motile spirilla were found in the blood in large numbers. Blood from one animal, taken during the attack, induced a similar febrile paroxysm when inoculated into another of the same species—relapses, such as characterize the disease in man, were not observed. One attack did not preserve the animals experimented upon from a similar attack when they were again inoculated after an interval of a few days. Soudakewitch (1891) has made successful inoculation experiments in monkeys, and has shown that in monkeys from which the spleen has previously been removed the spirilla continue to multiply very abundantly in the blood and the disease has a fatal termination, whereas in monkeys from which the spleen has not been removed the spirilla disappear from the blood within a few days after the access of the febrile paroxysm and the animal recovers.

SPIRILLUM ANSERUM.

Synonym.—Spirochæta anserina (Sakharoff).

Obtained by Sakharoff (1890) from the blood of geese affected by a fatal form of septicæmia due to this spirillum. This disease prevails among geese in Caucasia, especially in swampy regions, appearing annually and destroying a large number of the domestic geese.

Morphology.—Resembles the spirillum of relapsing fever. The long and flexible spiral filaments, when the disease is at its height, are often seen in interlaced masses, around the margins of which radiate single filaments which by their movements cause the whole mass to change its place, as if it were a single organism. These masses are sometimes so large that a single one occupies the entire field of the microscope.

Stains with the usual aniline colors.

Biological Characters.—An *aërobic, motile* spirillum. Not cultivated in artificial media. The movements are very active, resembling those of Spirillum Obermeieri, but cease in an hour or two in preparations made from the blood of geese containing it.

Pathogenesis.—A small quantity of blood from an infected goose inoculated into a healthy animal of the same species induces the disease after a period of incubation of four to five days. The infected goose ceases to eat, becomes apathetic, remaining in one place, and usually dies at the end of a week ; the temperature is increased, and in some cases there is diarrhœa. The spirilla are found in the blood at the outset of the malady, but after death they are not seen either in the blood or in the various organs. The heart and the liver are found to have undergone a fatty degeneration, and yellowish, cheesy granules the size of a millet seed are seen upon the surface of these organs. The spleen is soft and easily broken up by the fingers.

Inoculations into chickens and pigeons were without result; in one chicken the spirilla were found in the blood on the fourth day after inoculation, but the fowl recovered.

SPIRILLUM CHOLERÆ ASIATICÆ.

Synonyms.—Spirillum ("bacillus") of cholera; Comma bacillus of Koch; Kommabacillus der Cholera Asiatica; Bacille-virgule cholérigène.

Discovered by Koch (1884) in the excreta of cholera patients and in the contents of the intestine of recent cadavers.

The researches of Koch, made in Egypt and in India (1884), and subsequent researches by bacteriologists in various parts of the world, show that this spirillum—so-called "comma bacillus"—is constantly present in the contents of the intestine of cholera patients during the height of the disease, and that it is not found in the contents of the intestine of healthy persons or of those suffering from other diseases than cholera. The etiological relation of this spirillum to Asiatic cholera is now generally admitted by bacteriologists.

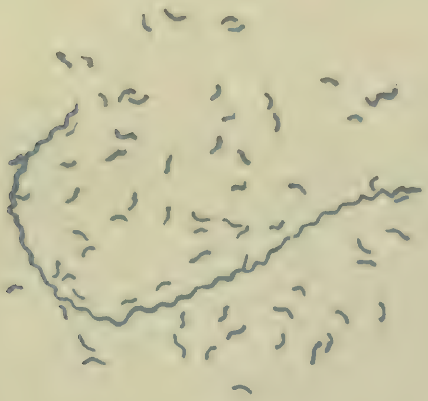


FIG. 172.

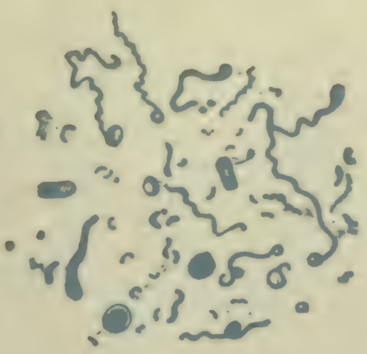


FIG. 173.

FIG. 172.—Spirillum choleræ Asiaticæ. × 1,000. From a photomicrograph. (Koch.)
FIG. 173.—Spirillum choleræ Asiaticæ, involution forms. × 700. (Van Ermengem.)

Morphology.—Slightly curved rods with rounded ends, from 0.8 to 2 μ in length and about 0.3 to 0.4 μ in breadth. The rods are usually but slightly curved, like a comma, but are occasionally in the form of a half-circle, or two united rods curved in opposite directions may form an S-shaped figure. Under certain circumstances the curved rods grow out into long, spiral filaments, which may consist of numerous spiral turns, and in hanging-drop cultures the S-shaped figures may also be seen to form the commencement of a spiral; in stained preparations the spiral character of the long filaments is often obliterated, or nearly so. When development is very rapid the short, curved rods or S-shaped spirals only are seen; but in hanging-drop cultures, or in media in which the development is retarded by an unfavorable temperature, the presence of a little alcohol, etc., the long, spiral filaments are quite numerous, and bacteriologists generally agree that the so-called "comma bacillus" is really only a fragment of a true spirillum. By Löffler's method of staining the rods may be seen to have a single terminal flagellum. In old cultures the bacilli frequently lose their characteristic form and become variously swollen and distorted—involution forms. Hueppe has described the appearance of spherical bodies in the course of the spiral filaments, which he believes to be reproductive elements—so-called arthrospores.

Stains with the aniline colors usually employed, but not as quickly as many other bacteria; an aqueous solution of fuchsin is the most reliable staining agent; is decolorized by iodine solution—Gram's method. Sections may be stained with Löffler's solution.

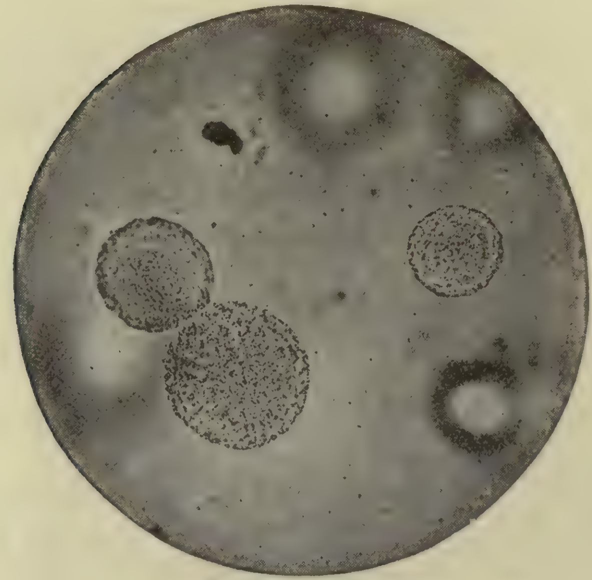


FIG. 174.

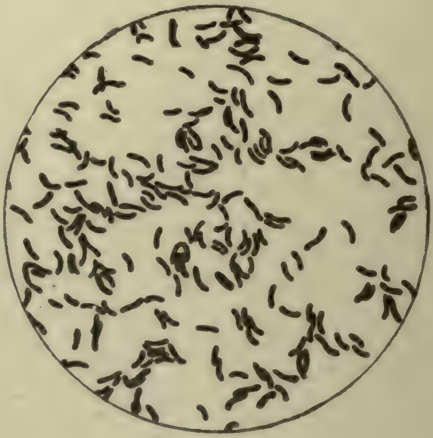


FIG. 175.

FIG. 174.—Spirillum choleræ Asiaticæ; colonies upon gelatin plate, end of thirty hours. × 100. Photograph by Fränkel and Pfeiffer.

FIG. 175.—Spirillum choleræ Asiaticæ, from a gelatin culture. × 1,000. From a photomicrograph. (Fränkel and Pfeiffer.)

Biological Characters.—An *aërobic* (facultative anaërobic), *liquefying, motile* spirillum. Grows in the usual culture media at the room temperature—more rapidly in the incubating oven. Does not grow at a temperature above 42° or below 14° C. Does not form endogenous spores (forms arthrospores, according to Hueppe ?).

In *gelatin plate cultures*, at 22° C., at the end of twenty-four hours small, white colonies may be perceived in the depths of the gelatin; these grow towards the surface and cause liquefaction of the gelatin in the form of a funnel which gradually increases in

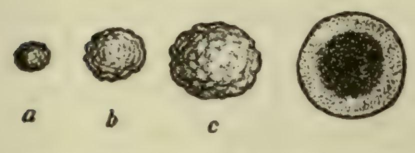


FIG. 176.—Colonies of the cholera spirillum; *a*, end of twenty hours; *b*, end of thirty hours; *c*, end of forty-eight hours; *d*, after liquefaction of the gelatin. (Flügge.)

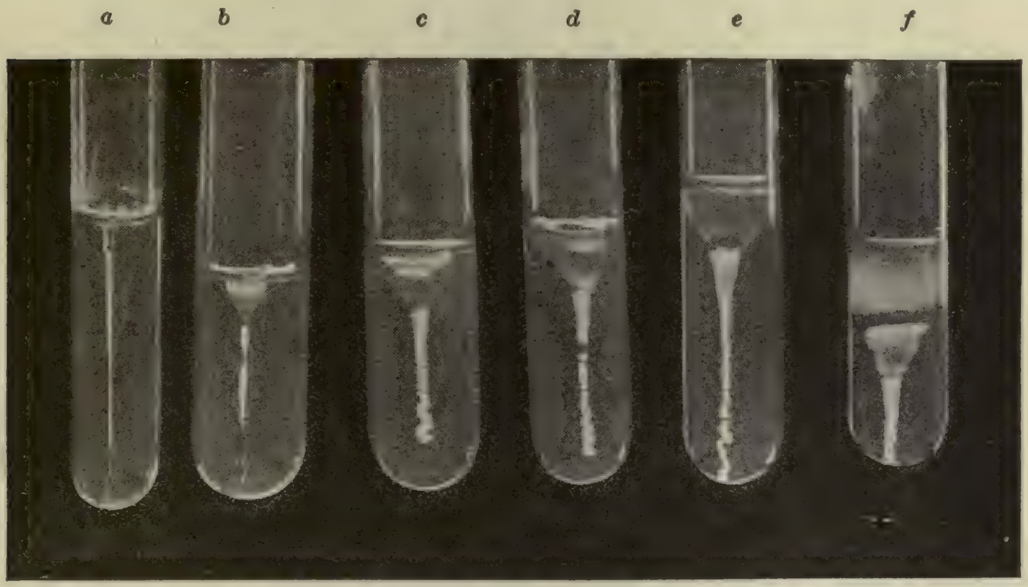


FIG. 177.—Spirillum choleræ Asiaticæ; *a*, one day old; *b*. three days old; *c*, four days old; *d*, five days old; *e*, seven days old; *f*, 10 days old. From photographs by Koch.

depth, and at the bottom of which is seen the colony in the form of a small, white mass; as a result of this the plates on the second or third day appear to be perforated with numerous small holes; later the gelatin is entirely liquefied. Under a low power the young colonies, before liquefaction has commenced, present a rather characteristic appearance; they are of a white or pale-yellow color, and have a more or less irregular outline, the margins being rough and uneven; the texture is coarsely granular, and the surface looks as if it were covered with little fragments of broken glass, while the colony has a shining appearance; when liquefaction commences an ill-defined halo is first seen to surround the granular colony, which by transmitted light has a peculiar roseate hue. In *stab cultures* in nutrient gelatin development occurs all along the line of inoculation, but liquefaction of the gelatin first occurs only near the surface; on the second day, at 22° C., a short funnel is formed which has a comparatively narrow mouth, and the upper portion of which contains air, while just below this is a whitish, viscid mass; later the funnel increases in depth and diameter, and at the end of from four to six days may reach the edge of the test tube; in from eight to fourteen days the upper two-thirds of the gelatin is completely liquefied. Owing to the slight liquefaction which occurs along the line of growth during the first three or four days, the central mass which had formed along the line of inoculation settles down as a curled or irregularly bent, yellowish-white thread in the lower part of a slender tube filled with liquefied gelatin, the upper part of which widens out and is continuous with the funnel above. Upon the surface of *nutrient agar* a moist, shining, white layer is formed along the line of inoculation—impfstrich. *Blood serum* is slowly liquefied by this spirillum. Upon the surface of cooked *potato,* in the incubating oven, a rather thin and semi-transparent brown or grayish-brown layer is developed. In *bouillon* the development is rapid and abundant, especially in the incubating oven ; the fluid is only slightly clouded, but the spirilla accumulate at the surface, forming a wrinkled membranous layer. *Sterilized milk* is also a favorable culture medium. In general this spirillum grows in any liquid containing a small quantity of organic pabulum and having a slightly alkaline reaction. An acid reaction of the culture medium prevents its development, as a rule, but it has the power of gradually accommodating itself to the presence of vegetable acids, and grows upon potatoes—in the incubator only—which have a slightly acid reaction. Abundant development occurs in bouillon which has been diluted with eight or ten parts of water, and the experiments of Wolffhügel

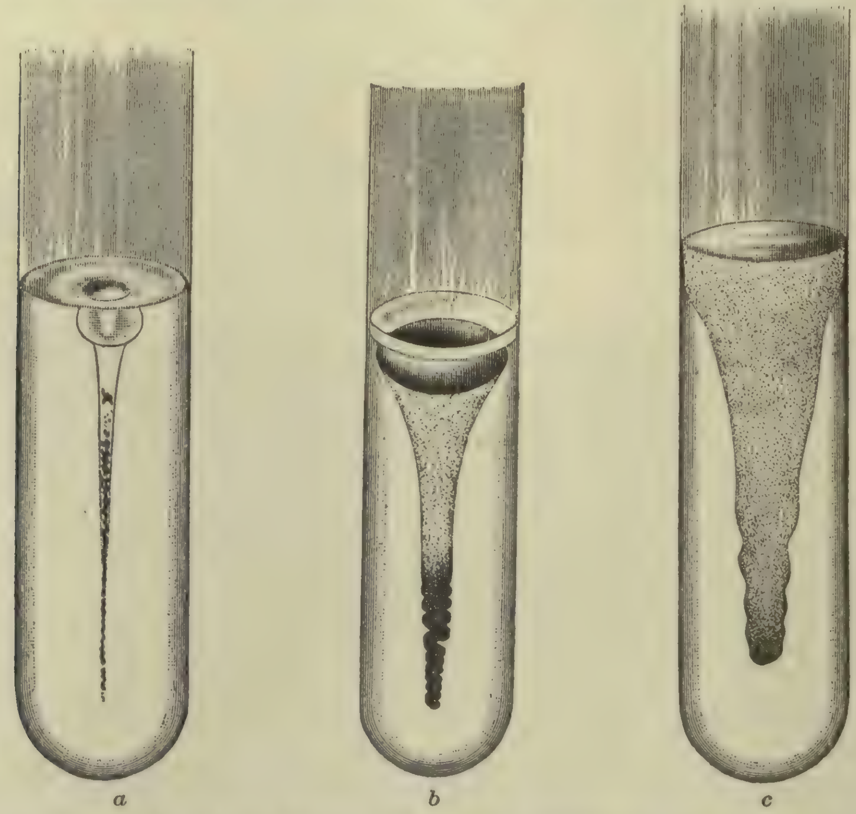


FIG. 178.—Cultures in nutrient gelatin, at the room temperature (16° to 18° C.), at the commencement of the fourth day; *a*, Spirillum choleræ Asiaticæ; *b*, Spirillum tyrogenum; *c*, Spirillum of Finkler and Prior. (Baumgarten.)

and Riedel show that it also multiplies to some extent in sterilized river or well water, and that it preserves its vitality in such water for several months. But in milk or water which contains other bacteria it dies out in a few days. Gruber and Schottelius have shown, however, that in bouillon which is greatly diluted the cholera spirillum may take the precedence of the common saprophytic bacteria, and that they form upon the surface of such a medium the characteristic wrinkled film. Koch found in his early investigations that rapid multiplication may occur upon the surface of moist linen, and also demonstrated the presence of this spirillum in the foul water of a "tank" in India which was used by the natives for drinking purposes. In the experiments of Bolton (1886) the cholera spirillum was found to multiply abundantly in distilled water to which bouillon was added in the proportion of fifteen to twenty-five parts in one thousand.

The thermal death-point of the cholera spirillum in recent cultures in flesh-peptone-gelatin, as determined by the writer (1887), is 52° C., the time of exposure being four minutes ; a few colonies only developed after exposure to a temperature of 50° for ten minutes. In Kitasato's experiments (1889) ten or even fifteen minutes' exposure to a temperature of 55° C. was not always successful in destroying the vitality of the spirillum, although in certain cultures exposure to 50° for fifteen minutes was successful. He was not, however, able to find any difference between old and recent cultures as regards resistance to heat or to desiccation. In a moist condition this spirillum retains its vitality for months—as much as nine months in agar and about two months in liquefied gelatin. It is quickly destroyed by desiccation, as first determined by Koch, who found that it did not grow after two or three hours when dried in a thin film on a glass cover. In Kitasato's experiments (1889) the duration of vitality was found to vary from a few hours to thirteen days, the difference depending largely upon the thickness of the film. When dried upon silk threads they may retain their vitality for a considerably longer time (Kitasato). Very numerous experiments have been made to determine the amount of various disinfecting agents required to destroy the vitality of this microörganism. We give below the results recently reported by Boer (1890), whose experiments were made in Koch's laboratory. Experiments upon a culture in bouillon kept for twenty-four hours in the incubating oven, time of exposure two hours : hydrochloric acid, 1 : 1,350 ; sulphuric acid, 1 : 1,300 ; caustic soda, 1 : 150 ; ammonia, 1 : 350 ; mercuric cyanide, 1 : 60,000 ; gold and sodium chloride, 1 : 1,000 ; silver nitrate, 1 : 4,000; arsenite of soda, 1 : 400 ; malachite green, 1 : 5,000 ; methyl violet, 1 : 1,000 ; carbolic acid, 1 : 400 ; creolin, 1 : 3,000 ; lysol, 1 : 500. In

Bolton's experiments (1887) mercuric chloride was effective in two hours in the proportion of 1 : 10,000 ; sulphate of copper, 1 : 500.

The low thermal death-point and comparatively slight resisting power for desiccation and chemical agents indicate that this spirillum does not form spores, and most bacteriologists agree that this is the case. Hueppe, however, has described a mode of spore formation which is different from that which occurs among the bacilli, viz., the formation of so-called arthrospores ; these are said to be developed in the course of the spiral threads, not as endogenous refractive spores, but as spherical bodies which have a somewhat greater diameter than the filament and are somewhat more refractive. This mode of spore formation has not been observed by Kitasato and other bacteriologists who have given attention to the question, and cannot be considered as established. In competition with the ordinary putrefactive bacteria the cholera spirillum soon disappears, and, as determined by Neffelman and by Kitasato, they only survive for a few days when mixed with normal fæces.

A test for the presence of the cholera spirillum has been found by Bujwid and by Dunham in the reddish-violet color produced in bouillon cultures containing peptone, or in cultures in nutrient gelatin, when a small quantity of sulphuric acid is added to the culture. According to Fränkel, this test serves to distinguish it from the ordinary bacteria of the intestine and from the Finkler-Prior spirillum, but not from Metschnikoff's spirillum ("vibrio"). The reaction is shown by bouillon cultures which have been in the incubating oven for ten or twelve hours, and by gelatin cultures in which liquefaction has occurred. The sulphuric acid used should be quite pure ; the color quickly appears and is reddish-violet or purplish-red. According to Salkowski, the red color is due to the well-known indol reaction, which in cultures of the cholera spirillum is exceptionally intense and rapid in its development. A test which is said to distinguish cultures of the cholera spirillum from the spirillum of Deneke and that of Finkler-Prior, has been proposed by Cahen. This consists in adding a solution of litmus to the bouillon and in making the culture at 37° C. The cholera cultures show on the following day a decoloration which does not occur at this temperature with the other spirilla named.

For determining as promptly as possible whether certain suspected excreta contain cholera spirilla, a little of the material may be used to inoculate greatly diluted bouillon, gelatin plates being made at the same time. At the end of ten or twelve hours the cholera spirillum, if present, will already have formed a characteristic wrinkled film upon the surface ; a little of this should be used to start a new culture in diluted bouillon, and a series of gelatin plates made from it, after which the color test may be applied. The result of this, in connection with the morphology of the microörganisms forming the film and the character of growth in the gelatin plates, will establish the diagnosis if the cholera spirillum is present in considerable numbers. If but few are present in the original material it may be necessary to make two or more series of plates and bouillon cultures before a pure culture can be obtained and a positive diagnosis made.

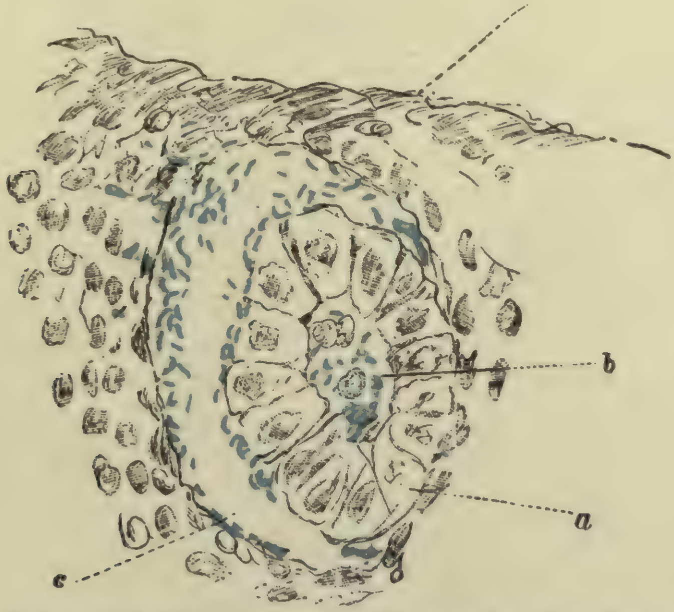


FIG. 179.—Section through mucous membrane of intestine from cholera cadaver; a tubular gland (*a*) is cut obliquely; in the interior of this (*b*), and between the epithelial and basement membrane, are numerous spirilla. × 600. (Flügge.)

The spirillum is not found in the blood or in the various organs of individuals who have succumbed to an attack of cholera, but it is constantly found in the alvine discharges during life and in the contents of the intestine examined immediately after death; frequently in almost a pure culture in the colorless "rice-water" discharges. It is evident, therefore, that if we accept it as the etiological agent in this disease, the morbid phenomena must be ascribed to the absorption of toxic substances formed during its multiplication in the intestine. In cases which terminated fatally after a very brief sickness Koch found but slight changes in the mucous membrane of the intestine, which was slightly swollen and reddened; but in more protracted cases the follicles and Peyer's patches were reddened around their margins, and an invasion of the mucous membrane by the "comma bacilli" was observed in properly stained sections; they penetrated especially the follicles of Lieberkühn, and in some cases were seen between the epithelium and basement membrane. As a rule, the spirillum is not present in vomited matters, but Koch found it in small numbers in two cases and Nicati and Rietsch in three. In about one hundred cases in which Koch examined the excreta, or the contents of the intestine of recent cadavers, during his stay in Egypt, in India, and in Toulon, his "comma bacillus" was constantly found, and other observers have fully confirmed him in this particular—Nicati and Rietsch in thirty-one cases examined at Marseilles; Pfeiffer, twelve cases in Paris; Schottelius in cases examined in Turin; Ceci in Genoa, etc. On the other hand, very numerous control experiments made by Koch and others show that it is not present in the alvine discharges of healthy persons or in the contents of the intestine of those who die from other diseases. In the writer's extended bacteriological studies of the excreta, and contents of the intestine of cadavers, in yellow fever, he has not once encountered any microörganism resembling the cholera spirillum.

As none of the lower animals are liable to contract cholera during the prevalence of an epidemic, or as a result of the ingestion of food contaminated with choleraic excreta, we have no reason to expect that pure cultures of the spirillum introduced by subcutaneous inoculation or by the mouth will give rise in them to a typical attack of cholera. Moreover, it has been shown by experiment that this spirillum is very sensitive to the action of acids, and is quickly destroyed by the acid secretions of the stomach, of man or the lower animals, when the functions of this organ are normally performed. By a special method of procedure, however, Nicati and Rietsch, and Koch, have succeeded in producing in guinea-pigs choleraic symptoms and death. The first-named investigators injected cultures of the spirillum into the duodenum, after first ligating the biliary duct; the animals experimented upon died, and the intestinal contents contained the spirillum in large numbers. The fact that this procedure involves a serious operation which alone might be fatal, detracts from the value of the results obtained. Koch's experiments on guinea-pigs are more satisfactory, and, having been fully controlled by comparative experiments, show that the "comma bacillus" is pathogenic for these animals when introduced in a living condition into the intestine. This was accomplished by first neutralizing the contents of the stomach with a solution of carbonate of soda—five cubic centimetres of a five-per-cent solution, injected into the stomach through a pharyngeal catheter. For the purpose of restraining intestinal peristalsis the animal also receives, in the cavity of the abdomen, a tolerably large dose of laudanum—one gramme tincture of opium to two hundred grammes of body weight. The animals are completely narcotized by this dose for about half an hour, but recover from it without showing any ill effects. Soon after the administration of the opium a bouillon culture of the cholera spirillum is injected into the stomach through a pharyngeal catheter. As a result of this procedure the animal shows an indisposition to eat and other signs of sickness, its posterior extremities become weak and apparently paralyzed, and, as a rule, death occurs within forty-eight hours. At the autopsy the small intestine is found to be congested and is filled with a watery fluid containing the spirillum in great numbers. Comparatively large quantities of a pure culture injected into the abdominal cavity of rabbits or of mice often produce a fatal result within two or three hours ; and Nicati and Rietsch have obtained experimental evidence of the pathogenic power of filtered cultures not less than eight days old. The most satisfactory evidence that this spirillum is able to produce cholera in man is afforded by an accidental infection which occurred in Berlin (1884), in the case of a young man who was one of the attendants at the Imperial Board of Health when cholera cultures were being made for the instruction of students. Through some neglect the spirillum appears to have been introduced into his intestine, for he suffered a typical attack of cholera, attended by thirst, frequent watery discharges, cramps in the extremities, and partial suppression of urine. Fortunately he recovered ; but the genuine nature of the attack was shown by the symptoms and by the abundant presence of the "comma bacillus" in the colorless, watery discharges from his bowels. Nicati and Rietsch observed a certain degree of attenuation in the pathogenic power of the spirillum after it had been cultivated for a considerable time at 20° to 25° C. ; and the observation has since been made that cultures which have been kept up from Koch's original stock have no longer the primitive pathogenic potency.

Cunningham, as a result of researches made in Calcutta (1891), arrives at the conclusion that Koch's "comma bacillus" cannot be accepted as the specific etiological agent in this disease. This conclusion is based upon the results of his own bacteriological studies, which may be summed up as follows : *First*, in many undoubted cases of cholera he has failed to find comma bacilli. *Second*, in one case he found three different species. *Third*, in one case the reaction with acids could not be obtained. From sixteen cases in which Cunningham made cultures he obtained ten different varieties of comma bacilli, the characters of which he gives in his published report. It may be that in India, which appears to be the permanent habitat of the cholera spirillum, many varieties of this microörganism exist ; but extended researches made in the laboratories of Europe show that Cunningham is mistaken in supposing that spirilla resembling Koch's "comma bacillus" are commonly present in the intestine of healthy persons. The view advocated is that during the attack these spirilla are found in increased numbers because conditions are more favorable for their development, but that they have no etiological import. The writer would remark that, in very extended researches made in the United States and in Cuba, he has never found any microörganism resembling Koch's cholera spirillum in the fæces of patients with yellow fever or of healthy individuals, or in the intestinal contents of yellow-fever cadavers.

SPIRILLUM OF FINKLER AND PRIOR.

Synonym.—Vibrio proteus.

Obtained by Finkler and Prior (1884) from the fæces of patients with cholera nostras, after allowing the dejecta to stand for some days. Subsequent researches have not sustained the view that this spirillum is the specific cause of cholera morbus.

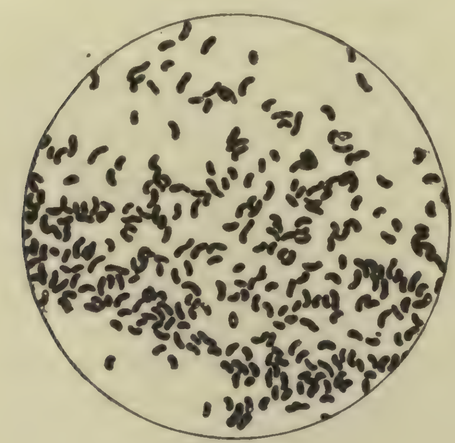


FIG. 180.

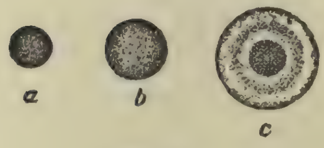


FIG. 181.

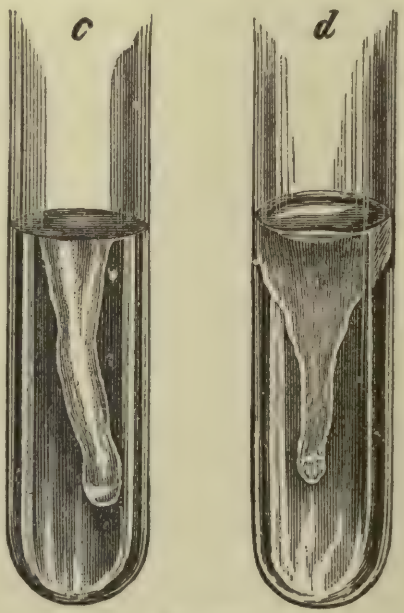


FIG. 182.

FIG. 180.—Spirillum of Finkler and Prior, from a gelatin culture. × 1,000. From a photomicrograph. (Fränkel and Pfeiffer.)

FIG. 181.—Spirillum of Finkler and Prior; colonies upon gelatin plate; *a*, end of sixteen hours; *b*, end of twenty-four hours; *c*, end of thirty-six hours. × 80. (Flügge.)

FIG. 182.—Spirillum of Finkler and Prior; culture in nutrient gelatin; *c*, two days old; *d*, four days old. (Flügge.)

Morphology.—Resembles the spirillum of Asiatic cholera, but the curved segments ("bacilli") are somewhat longer and thicker and not so uniform in diameter, the central portion being usually thicker than the somewhat pointed ends; forms spiral filaments, which are not as numerous, and are usually shorter than those formed by the cholera spirillum. In unfavorable media involution forms are common—large oval, spherical, or spindle-shaped cells, etc. Has a single flagellum at one end of the curved segments, which is from one to one and one-half times as long as these.

Stains with the usual aniline colors—best with an aqueous solution of fuchsin.

Biological Characters.—An *aërobic* and *facultative anaërobic, liquefying, motile* spirillum. Spore formation not demonstrated. Grows in the usual culture media at the room temperature. Upon gelatin plates small, white, punctiform colonies are developed at the end of twenty-four hours, which under the microscope are seen to be finely granular and yellowish or yellowish-brown in color; liquefaction of the gelatin around these colonies progresses rapidly, and at the end of forty-eight hours is usually complete in plates where they are numerous. Isolated colonies on the second day form saucer-shaped depressions in the gelatin the size of lentils, having a sharply defined border. In *gelatin stab cultures* liquefaction progresses much more rapidly than in similar cultures of the cholera spirillum, and a stocking-shaped pouch of liquefied gelatin is already seen on the second day, which rapidly increases in dimensions, so that by the end of a week the gelatin is usually completely liquefied; upon the surface of the liquefied medium a whitish film is seen. Upon *agar* a moist, slimy layer, covering the entire surface, is quickly developed. The growth in *blood serum* is rapid and causes liquefaction of the medium. Upon *potato* this spirillum grows at the room temperature and produces a slimy, grayish-yellow, glistening layer, which soon extends over the entire surface. The cholera spirillum does not grow upon potato at the room temperature. The cultures of the Finkler-Prior spirillum give off a tolerably strong putrefactive odor, and, according to Buchner, in media containing sugar an acid reaction is produced as a result of their development. They have a greater resistance to desiccation than the cholera spirillum.

Pathogenesis.—Pathogenic for guinea-pigs when injected into the stomach by Koch's method, after previous injection of a solution of carbonate of soda, but a smaller proportion of the animals die from such injections (Koch). At the autopsy the intestine is pale, and its watery contents, which contain the spirilla in great numbers, have a penetrating, putrefactive odor.

SPIRILLUM TYROGENUM.

Synonyms.—Spirillum of Deneke; Käsespirillen.

Obtained by Deneke (1885) from old cheese.

Morphology.—Curved rods and long, spiral filaments resembling the spirilla of Asiatic cholera. The diameter of the curved segments is somewhat less than that of the cholera spirillum, and the turns in the spiral filaments are lower and closer together. The diameter of the "commas" is uniform throughout, so that this spirillum more closely resembles the cholera spirillum than does that of Finkler and Prior.

Stains with the usual aniline colors—best with an aqueous solution of fuchsin.

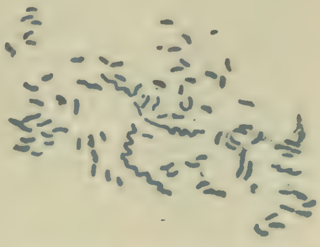


FIG. 183.—Spirillum tyrogenum. × 700. (Flügge.)

Biological Characters.—An *aërobic* and *facultative anaërobic, liquefying, motile* spirillum. Spore formation not demonstrated. Grows in the usual culture media at the room temperature—more rapidly than the cholera spirillum and less so than that of Finkler and Prior. Upon *gelatin plates* small, punctiform colonies are developed, which on the second day are about the size of a pin's head and have a yellowish color; under the microscope they are seen to be coarsely granular, of a yellowish-green color in the centre and paler towards the margins. The outlines of the colonies are sharply defined at first, but later, when liquefaction has commenced, the sharp contour is no longer seen. At first liquefaction of the gelatin causes funnel-shaped cavities resembling those formed by the cholera spirillum, but liquefaction is more rapid. In *gelatin stab cultures* liquefaction occurs all along the line of puncture, and the spirilla sink to the bottom of the liquefied gelatin in the form of a coiled mass, while a thin, yellowish layer forms upon the surface; complete liquefaction usually occurs in about two weeks. Upon the surface of *agar* a thin, yellowish layer forms along the impfstrich. Upon *potato*, at a temperature of 37° C., a thin, yellow layer is usually developed (not always—Eisenberg); this contains, as a rule, beautifully formed, long, spiral filaments.

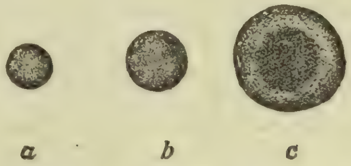


FIG. 184.—Spirillum tyrogenum; colonies in gelatin plate; *a*, end of sixteen hours; *b*, end of twenty-four hours; *c*, end of thirty-six hours. × 80. (Flügge.)

Pathogenesis.—Pathogenic for guinea-pigs when introduced into the stomach by Koch's method; three out of fifteen animals treated in this way succumbed.

SPIRILLUM METSCHNIKOVI.

Synonym.—Vibrio Metschnikovi (Gameléia).

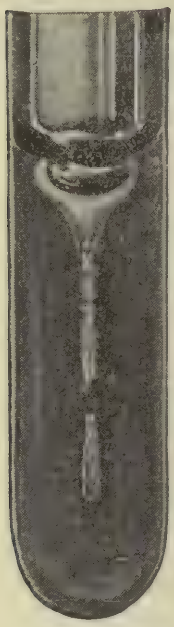


FIG. 185.—Spirillum Metschnikovi; culture in nutrient gelatin, end of forty-eight hours. From a photograph. (Fränkel and Pfeiffer.)

Obtained by Gameléia (1888) from the intestinal contents of chickens dying of an infectious disease which prevails in certain parts of Russia during the summer months, and which in some respects resembles fowl cholera. The experiments of Gameléia show that the spirillum under consideration is the cause of the disease referred to, which he calls gastro-enteritis cholerica.

Morphology.—Curved rods with rounded ends, and spiral filaments; the curved segments are usually somewhat shorter, thicker, and more decidedly curved than the "comma bacillus" of Koch. The size differs very considerably in the blood of inoculated pigeons, the diameter being sometimes twice as great as that of the cholera spirillum, and at others about the same. A single, long, undulating flagellum may be seen at one extremity of the spiral filaments or curved rods in properly stained preparations.

Stains with the usual aniline colors, but not by Gram's method.

Biological Characters.—An *aërobic* (facultative anaërobic ?), *liquefying*, *motile* spirillum. According to Gamaléia, endogenous spores are formed by this spirillum; but Pfeiffer does not confirm this observation, and it must be considered extremely doubtful in view of the slight resistance to heat—killed in five minutes by a temperature of 50° C. Grows in the usual culture media at the room temperature. Upon *gelatin plates* small, white, punctiform colonies are developed at the end of twelve to sixteen hours; these rapidly increase in size and cause liquefaction of the gelatin, which is, however, much more rapid with some than with others. At the end of three days large, saucer-like areas of liquefaction may be seen resembling that produced by the Finkler-Prior spirillum and the contents of which are turbid, while other colonies have produced small, funnel-shaped cavities filled with transparent, liquefied gelatin and resembling colonies of the cholera spirillum of the same age. Under the microscope the larger liquefied areas are seen to contain yellowish-brown granular masses which are in active movement, and the margins are surrounded by a border of radiating filaments. In *gelatin stab cultures* the growth resembles that of the cholera spirillum, but the development is more rapid. Upon *agar*, at 37° C., a yellowish layer resembling that formed by the cholera spirillum is quickly developed. Upon *potato* no growth occurs at the room temperature, but at 37° C. a yellowish-brown or chocolate-colored layer is formed, which closely resembles that produced by the cholera spirillum under the same circumstances. In *bouillon*, at 37° C., development is extremely rapid, and the liquid becomes clouded and opaque, having a grayish-white color, while a thin, wrinkled film forms upon the surface. When muriatic or sulphuric acid is added to a culture in peptonized bouillon a red color is produced similar to that produced in cultures of the cholera spirillum, and even more pronounced. In *milk*, at 35° C., rapid development occurs, and the milk is coagulated at the end of a week; the precipitated casein accumulates at the bottom of the tube in irregular masses and is not redissolved. The milk acquires a strongly acid reaction and the spirilla quickly perish.

Pathogenesis.—Pathogenic for chickens, pigeons, and guinea-pigs; rabbits and mice are refractory except for very large doses. Chickens suffering from the infectious disease caused by this spirillum remain quiet and somnolent, with ruffled feathers; they have diarrhœa; the temperature is not elevated above the normal, as is the case in chicken cholera. At the autopsy the most constant appearance is hyperæmia of the entire alimentary canal. A grayish-yellow liquid, more or less mixed with blood, is found in considerable quantity in the small intestine; the spleen is not enlarged and the organs generally are normal in appearance. In adult chickens the spirillum is not found in the blood, but in young ones its presence may be verified by the culture method and by inoculation into pigeons, which die in from twelve to twenty hours after being inoculated with two to four cubic centimetres. The pathological appearances in pigeons correspond with those found in chickens, but usually the spirillum is found in great numbers in blood taken from the heart. A few drops of a pure culture inoculated subcutaneously in pigeons or injected into the muscles cause their death in eight to twelve hours. Gameléia claims that the virulence of cultures is greatly increased by successive inoculations in pigeons, but Pfeiffer has shown that very minute doses are fatal to pigeons and that no decided increase of virulence occurs as a result of successive inoculations. According to Gameléia, chickens may be infected by giving them food contaminated with the cultures of the spirillum, but pigeons resist infection in this way. Guinea-pigs usually die in from twenty to twenty-four hours after receiving a subcutaneous inoculation; at the autopsy an extensive subcutaneous œdema is found in the vicinity of the point of inoculation, and a superficial necrosis may be observed; the blood and the organs generally contain the "vibrio" in great numbers, showing that the animals die from general infection—acute septicæmia. When infection occurs in these animals by way of the stomach the intestine will be found highly inflamed and its liquid contents will contain numerous spirilla.

Gameléia has shown that pigeons and guinea-pigs may be made immune by inoculating them with sterilized cultures of the spirillum—sterilized by heat at 100° C. Old cultures contain more of the toxic substance than those of recent date. Thus two to three cubic centimetres of a culture twenty days old will kill a guinea-pig when injected subcutaneously, while five cubic centimetres of a culture five days old usually fail to do so. According to Pfeiffer, old cultures have a decidedly alkaline reaction, and their toxic power is neutralized by the addition of sulphuric acid.

Gameléia has claimed that by passing the cholera spirillum of Koch through a series of pigeons, by successive inoculation, its pathogenic power is greatly increased, and that when sterilized cultures of this virulent variety of the "comma bacillus" are injected into pigeons they become immune against the pathogenic action of the "vibrio Metschnikoff," and the reverse. Pfeiffer (1889), in an extended and carefully conducted research, was not able to obtain any evidence in support of this claim.

NOTES RELATING TO THE PATHOGENIC SPIRILLA.

Quite a number of spirilla have been obtained from various sources which resemble more or less closely the spirillum of Asiatic cholera. It appears probable that some of these are in fact varieties of Koch's "comma bacillus" which have undergone various modifications as a result of the conditions under which they have maintained their existence as saprophytes. Others are evidently essentially different, and have no very near relationship to the cholera spirillum. The principal points of difference between these recently described spirilla and Spirillum choleræ Asiaticæ are given in the following résumé, for which we are indebted to Dieudonné (1894).

"Since the outbreak of cholera in 1892, various vibrios have been described which resemble more or less closely the cholera vibrio. When these are tested as to their morphological characters, growth in peptone solutions, in gelatin and agar plates, cholera-red reaction, and pathogenic power, they may be divided, at the outset, into two groups: viz., such vibrios as show only a remote resemblance to the cholera vibrio, and therefore are easily differentiated from it, and such as present only minor differences or none at all that have been demonstrated.

"To the first group belongs the spirillum isolated by Russell from sea water—Spirillum marinum—which rapidly liquefies gelatin and does not grow at the body temperature. Rénon isolated from water, obtained at Billancourt, a vibrio which likewise quickly liquefies gelatin, but is not pathogenic for guinea-pigs, either by subcutaneous or intraperitoneal inoculation. Günther, in examining the Spree water, found a vibrio which, upon gelatin plates, formed circular colonies with smooth margins, very finely granular and of a brown color. This vibrio did not give the indol reaction, and all infection experiments gave a negative result. Günther named this saprophyte Vibrio aquatilis. About the same time (1892) Kiessling obtained from water, from Blankenese, a vibrio which presented similar characters and probably is identical with that of Günther. Weibel obtained from well-water a vibrio which liquefies gelatin more rapidly than the cholera vibrio; its pathogenic action was not tested. Bujwid (1893) isolated from Weichsel water a vibrio which at low temperatures (12° C.) grew almost the same as the cholera vibrio, but at higher temperatures was easily distinguished from it. Bujwid's assistant, Orlowski, found in a well at Lubin a very similar vibrio. Löffler (1893) obtained from the Peene water a vibrio which at 37° C. grows rapidly and liquefies gelatin very rapidly, like the Finkler-Prior spirillum. Fokker (1893), from water of the harbor at Gröningen, obtained a vibrio which rapidly liquefied gelatin and occasionally gave the indol reaction. Injections into the peritoneal cavity of mice and guinea-pigs gave a negative result. Fokker supposes that this is an attenuated cholera bacillus, because it forms the same ensyme as cholera bacteria, and when cultivated for three months its characters, especially its peptonizing power, had changed. Fischer (1893) found in the stools of a woman suffering from diarrhœa a vibrio which in gelatin cultures resembled that of Finkler and Prior. In bouillon and peptone solution it caused clouding and formation of a pellicle, but only gave a slight indol reaction. A portion of the mice inoculated subcutaneously had after a time abscesses, from the contents of which Fischer was able to cultivate his vibrio, which he named Vibrio helcogenes. Vogler (1893), in an extended series of examinations of fæces, found a vibrio which showed many points of resemblance to the cholera vibrio in its growth in gelatin. But it constantly gave a negative indol reaction, and was not pathogenic for guinea-pigs when injected into the peritoneal cavity. Bleisch obtained from the dejecta of a man who died with choleraic symptoms a bacterium which upon gelatin plates grew at first like the cholera bacillus, but was distinguished from it by many points of difference in other respects: short rods, sometimes bent, but never showing spiral forms. It gave the cholera-red reaction. Wolf (1883) obtained from cervical secretion, from a woman suffering from chronic endometritis, a comma-formed bacillus, which in its growth on gelatin plates resembled the cholera vibrio. The liquefaction was, however, much more rapid, a culture a day old being as far advanced as a cholera culture of three to four days. The addition of sulphuric acid to a bouillon culture caused a faint rose-red color, which upon standing changed to brown. The addition of sulphuric acid and potassium iodide paste did not cause a blue color, so there was no formation of nitrites. Bonhoff (1893), in water from Stolpe, in Pommerania, discovered two vibrios, one of which in the first twenty-four hours grew like the cholera vibrio, but did not give the cholera-red reaction. Out of four guinea-pigs inoculated one only died with cholera-like symptoms. The other vibrio gave the cholera-red reaction, but did not liquefy gelatin and was very inconstant as regards its pathogenic power. Zörkendörfer (1893) isolated a vibrio from the stools of a woman who died with choleraic symptoms, which at first grew upon gelatin plates like the cholera vibrio, but after the second day liquefied the gelatin very rapidly, so that it could no longer be taken for the same. The indol reaction was constantly absent, and it was not pathogenic for guinea-pigs, rabbits, or pigeons. Blackstein (1893) obtained from the water of the Seine a comma bacillus which resembled the cholera vibrio in many particulars, but was distinguished by the finer granulation and more opaque appearance of its colonies. Sanarelli (1893), by the use of special media, isolated from the water of the Seine and of the Marne no less than thirty-two vibrios, four of which resembled the cholera vibrio in giving the indol reaction. Three others gave the indol reaction after eight days; the remainder did not give it at all, or only very faintly. The vibrios which upon a first inoculation gave no results or only very slight evidence of pathogenic power, when carried through a series of animals caused a fatal infection. When a sterilized culture of the colon bacillus was injected at the same time death always occurred. Sanarelli believes that these vibrios must have had a common origin—from the dejecta of cholera patients. Fischer (1894) has described a number of vibrios from sea-water which are distinguished from the cholera vibrio especially by a preference for media containing sea-water. Finally, the vibrios found in water, referred to by Koch ('Ueber den augenblicklichen Stand der Cholera-diagnose,' *Zeitschr. für Hygiene*, Bd. xiv., page 319), belong here.

"Quite different from these is a second group of vibrios which in their investigation offered great and often almost insuperable difficulties for the differential diagnosis. Here, first of all, is the Vibrio Berolinensis, found by Neisser in August, 1893, and described by Rubner, Neisser, and Günther. This was isolated from water which had previously contained cholera vibrios, for which reason Dunbar considers it not impossible that this is a genuine cholera vibrio, somewhat changed perhaps by long-continued development in water. Neither in its morphology nor in its behavior in gelatin stick cultures, in milk and other media, could it be distinguished from the genuine comma bacillus; the indol reaction and pathogenic action upon guinea-pigs were the same; on the contrary, a differentiation was easily made in gelatin plate cultures. At the end of twenty-four hours it formed small, spherical, finely granular colonies, which at the end of forty-eight hours were not yet visible to the naked eye. Heider (1893) isolated from the water of the Donau canal a vibrio which he called Vibrio Danubicus. This resembles the cholera vibrio fully in its morphology. As a distinguishing character it was found that this vibrio, in thinly planted plates, forms flat, superficial colonies having irregularly rounded margins and other slight differences; also the pathogenic action upon mice inoculated subcutaneously, and the ease with which guinea-pigs are infected by way of the respiratory passages. It is worthy of note that the day after the sample was taken a man was taken sick with cholera who had worked on the Donau the day before—on the principal stream at a place far below the junction of the canal. Dunbar (1893) found vibrios in the Elbe, in the Rhine, in the Pegnitz, and in the Amstel at Amsterdam. These presented no decided characters by which he was able to differentiate them from the cholera vibrio. The most careful comparative investigations did not lead to the discovery of any points of difference which had not already been observed in genuine cholera cultures. Everything, therefore, indicated that these were genuine cholera bacilli, especially as these vibrios disappeared from the rivers when cholera ceased to prevail. It was first possible through an observation of Kutscher's to differentiate a portion of these water bacteria, and certain vibrios isolated from the discharges of persons suspected of having cholera from cultures of the cholera spirillum. In the presence of oxygen, at a suitable temperature, they give off a greenish-white phosphorescence.

"As phosphorescence has never been observed in undoubted cholera cultures, we can assert with tolerable certainty that such phosphorescent vibrios are not genuine cholera bacteria. But as this phosphorescent property was inconstant in thirty-eight out of sixty-eight cultures, Dunbar believes that some reserve must be exercised in accepting this as evidence that these are not genuine cholera vibrios. Maassen (1894) gives as a further distinguishing character of these phosphorescent vibrios the fact that they form a strong, usually wrinkled pellicle in bouillon, of proper alkalinity, containing glycerin or carbohydrates (cane sugar, lactose); also that in such media the formation of indol and a subsequent return to an alkaline reaction may be observed.

"As already stated, Sanarelli isolated from Seine water a considerable number of vibrios, and among them four—viz.: one from St. Cloud, Point-du-Jour, Gennevilliers No. 5, and Versailles (Seine), which after twenty-four hours gave a distinct indol reaction and were more or less pathogenic for guinea-pigs (the one from St. Cloud was also pathogenic for pigeons). Ivánoff (1893) describes a vibrio which he isolated from the fæces of a patient with typhoid fever. But as the discharges had been mixed with Berlin hydrant water, Ivánoff admits the possibility that his vibrio came from this water. It closely resembles the cholera vibrio, but is distinguished by its colonies in gelatin plates, which, at the end of twenty-four to thirty-six hours, in place of the usual coarse granulation of cholera colonies shows a distinct formation of filaments. Morphologically the vibrio is distinguished by a decided tendency to preserve the spiral form, and especially by its size. Celli and Santori (1893) describe a Vibrio romanus, which they isolated from twelve undoubted cases of cholera. This does not give the indol reaction, is not pathogenic for animals, and does not grow in bouillon or agar at 37° C. This is considered by the authors named an atypical variety of the cholera vibrio, especially as the distinguishing characters did not prove to be permanent. After eight months' cultivation the cultures gave the indol reaction, but the pathogenic power was still almost absent. Recently Chantemesse (1894) has described a vibrio which he found in the spring of 1894 during the cholera epidemic at Lisbon. This differed in many particulars from the genuine cholera vibrio, resembling more closely the vibrio of Finkler-Prior. As in the Lisbon epidemic, with a large number taken sick, only one death occurred, and in view of the results of the bacteriological examination, Chantemesse supposes this to have been an epidemic of cholera nostras. Finally, Pfuhl (1894) found a vibrio in the north harbor of Berlin which from its growth in gelatin and pathogenesis for pigeons he believes to be identical with Vibrio Metschnikovi."

To the list of vibrios above referred to as resembling more or less closely the cholera spirillum we must add those described by Cunningham (1894) and obtained by him from the discharges of cholera patients. He has described "thirteen distinct forms obtained from cases of cholera and one of non-choleraic origin."

Pfeiffer and Issaeff (1894) report that they have found a sensitive test for the differentiation of these vibrios in the specific character of cholera immunity. They found that guinea-pigs which were immunized against cholera infection have a lasting immunity, and that the serum of such immunized animals has a specific action in protecting against infection by genuine cholera vibrios only, while for other species it has no action different from that of the blood serum of normal animals. In all cases where the cholera serum acted specifically the vibrios were promptly destroyed, while in cases where this specific action was absent the injected vibrios multiplied rapidly and caused the death of the animal. By means of this method the vibrios isolated from water—the phosphorescent vibrios of Dunbar, Vibrio Danubicus, Cholera Massanah—are shown to be distinct species, while the vibrio of Ivánoff behaves like the genuine cholera vibrio. In a subsequent paper Pfeiffer reports the interesting fact that a trace of highly active cholera serum, added to a culture of the cholera spirillum, when injected into the peritoneal cavity of a guinea-pig, within a surprisingly brief time causes the destruction of the cholera vibrios; whereas no such effect is produced upon other species. A similar destruction occurs when cholera vibrios are injected into the abdominal cavity of immunized guinea-pigs. The researches of Dunbar (1894) indicate that Pfeiffer's test is not so reliable as he supposed; and also that phosphorescence cannot be relied upon for distinguishing similar water bacteria from genuine cholera vibrios. Rumpel has reported the fact that two undoubted cultures of the cholera spirillum, from different sources, after being passed through pigeons and cultivated for some time in artificial media, showed phosphorescence. One of these cultures was obtained originally from the discharges of Dr. Oergel, who was a victim to cholera from laboratory infection (case reported by Reincke, in the *Deutsche medicinische Wochenschrift*, No. 41, 1894). Another case of supposed laboratory infection, in which recovery occurred, is reported by Lazarus, in the *Berliner medicinische Wochenschrift*, 1893, page 1,241.

That cholera vibrios may be present in the alimentary canal of healthy individuals without giving rise to any symptoms of ill-health appears to be demonstrated. In support of this conclusion we quote as follows from a recent paper by Abel and Claussen:

"In Wehlau (East Prussia), in the autumn of 1894, seven cases of cholera occurred about the same time. The members of the family were at once isolated and their fæces examined almost daily. Of especial interest were seventeen individuals who belonged to families in which three fatal cases occurred. Of these seventeen persons, who were not sick at all or only had for a brief time a diarrhœa, thirteen had cholera vibrios in their discharges for a considerable time. As the table shows, many of these comma bacilli were not found in discharges every day, but were obtained again after being absent" (in the cultures) "for a day or two."

Abel and Claussen (1895), as a result of very extended experiments, arrive at the conclusion that cholera vibrios in fæces as a rule do not survive longer than twenty days, and often cannot be obtained after two or three days; exceptionally they were obtained in cultures at the end of thirty days—Karlinsky and Dunbar have reported finding them at the end of fifty-two days and four months. Karlinsky (1895) has also reported that upon woollen and linen goods, cotton batting and wool, which were soaked in the discharges of cholera patients and preserved from drying by being wrapped in waxed paper, the cholera vibrio retained its vitality for from twelve to two hundred and seventeen days.

The researches of Kasansky (1895) show that the cholera spirillum is not destroyed by a low temperature (−30 C.) and that it even resists repeated freezing and thawing—three or four times.

Behring and Ransom (1895) as a result of an extended experimental research, arrive at the conclusion that cholera cultures from which the bacteria have been removed have specific toxic properties, and cause symptoms similar to those which result from the introduction into guinea-pigs of the living bacteria; that from these filtered cultures a solid substance can be obtained having the same toxic properties, and that from susceptible animals which have been treated with this toxic substance a serum can be obtained which is active not only against the cholera poison, but against the cholera vibrio. These results support those previously reached by other bacteriologists and lead to the hope that a specific treatment of the disease may be successfully employed. The results obtained by Haffkine in India are favorable to the view that his method of prophylaxis, by the subcutaneous injection of virulent cholera cultures, has a real value.

PLATE IX.

Fig. 1.—Bacillus diphtheriæ (Klebs-Löffler) from culture on blood serum. Stained with Löffler's solution of methylene blue. × 1,000. Photomicrograph by oil lamp. (Borden.)

Fig. 2.—Micrococcus gonorrhœæ in urethral pus. Stained with Löffler's solution of methylene blue. × 1,000. Photomicrograph by oil lamp. (Borden.)

Fig. 3.—Bacillus tuberculosis in sputum. × 1,000. Photomicrograph by oil lamp. (Borden.)

Fig. 4.—Bacillus typhi abdominalis, from agar culture. × 1,000. Photomicrograph by oil lamp. (Borden.)

Fig. 5.—Streptococcus pyogenes (longus). × 1,000. Photomicrograph made at the Army Medical Museum by sunlight. (Gray.)

Fig. 6.—Bacillus mallei. × 1,000. Photomicrograph made at the Army Medical Museum by sunlight. (Gray.)

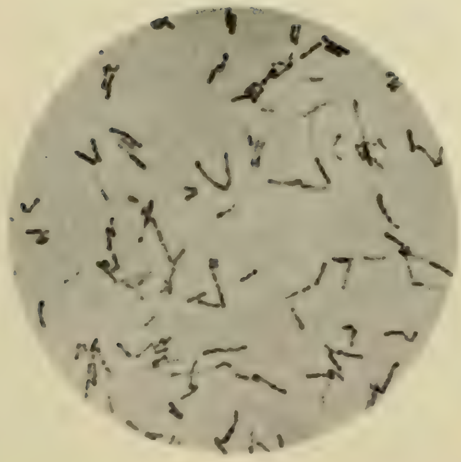


Fig. 1.

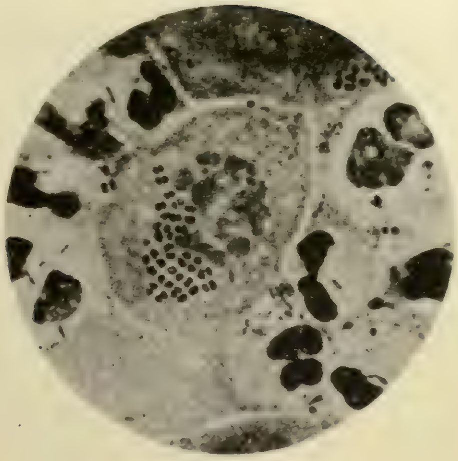


Fig. 2.

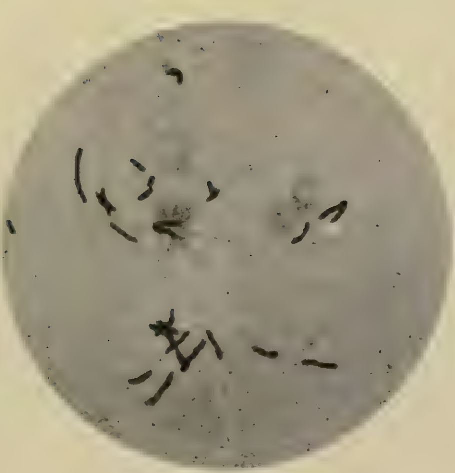


Fig. 3.

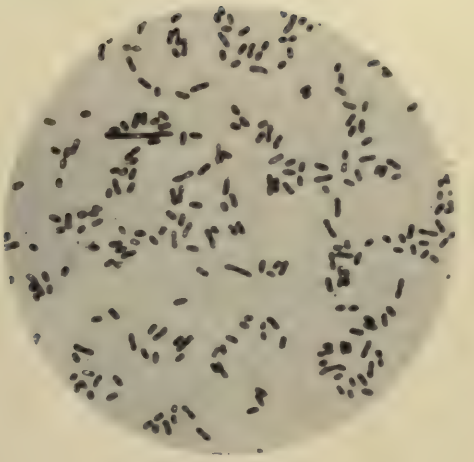


Fig. 4.

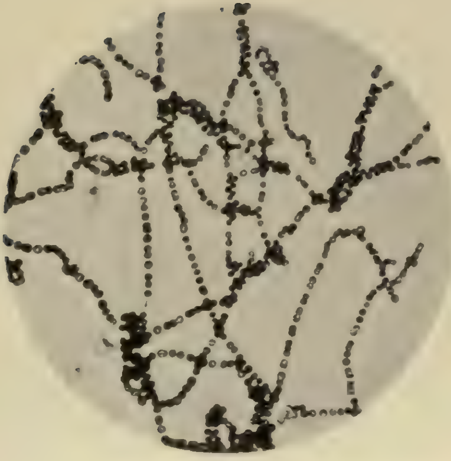


Fig. 5.

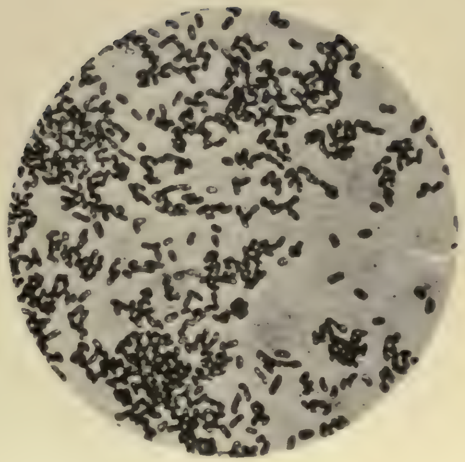


Fig. 6.

PATHOGENIC BACTERIA.

PART FOURTH.

SAPROPHYTES.

I. BACTERIA IN THE AIR. II. BACTERIA IN WATER. III. BACTERIA IN THE SOIL. IV. BACTERIA ON THE SURFACE OF THE BODY AND OF EXPOSED MUCOUS MEMBRANES. V. BACTERIA OF THE STOMACH AND INTESTINE. VI. BACTERIA OF CADAVERS AND OF PUTREFYING MATERIAL FROM VARIOUS SOURCES. VII. BACTERIA IN ARTICLES OF FOOD.

I.

BACTERIA IN THE AIR.

The saprophytic bacteria are found wherever the organic material which serves as their pabulum is exposed to the air under conditions favorable to their growth. The essential conditions are presence of moisture and a suitable temperature. The organic material may be in solution in water or in the form of moist masses of animal or vegetable origin, and the temperature may vary within considerable limits—0° to 70° C. But the species which takes the precedence will depend largely upon special conditions. Thus certain species multiply abundantly in water which contains comparatively little organic pabulum, and others require a culture medium rich in albuminous material or in carbohydrates; some grow at a comparatively low or high temperature, while others thrive only at a temperature of 20° to 40° C. or have a still more limited range; some require an abundant supply of oxygen, and others will not grow in the presence of this gas. Our statement that saprophytic bacteria are found wherever the organic material which serves as their pabulum is exposed to the air—under suitable conditions—relates to the fact that it is through the air that these bacteria are distributed and brought in contact with exposed material. It is a matter of common laboratory experience that sterilized organic liquids quickly undergo putrefactive decomposition when freely exposed to the air, and may be preserved indefinitely when protected from the germs suspended in the air by means of a cotton air filter. But the organic pabulum required for the nourishment of these bacteria is not found in the air in any considerable amount, and if they ever multiply in the atmosphere it must be under very exceptional conditions. Their presence is due to the fact that they are wafted from surfaces where they exist in a desiccated condition, and, owing to their levity, are carried by the wind to distant localities. But, under the law of gravitation, when not exposed to the action of currents of air they constantly fall again upon exposed surfaces, which, if moist, retain them, or from which, if dry, they are again wafted by the next current of air. Under these circumstances it is easy to understand why, as determined by investigation, more bacteria are found near the surface of the earth than at some distance above the surface, more over the land than over the ocean, more in cities with their dust-covered streets than in the country with its grass-covered fields.

Careful experiments have shown that bacteria do not find their way into the atmosphere from the surface of liquids, unless portions of the liquid containing them are projected into the air by some mechanical means, such as the bursting of bubbles of gas. Cultures of pathogenic bacteria freely exposed to the air in laboratories do not endanger the health of those who work over them; but if such a culture is spilled upon the floor and allowed to remain without disinfection, when it is desiccated the bacteria contained in it will form part of the dust of the room and might be dangerous to its occupants. Bacteria do not escape into the air from the surface of the fluid contents of sewers and cesspools, but changes of level may cause a deposit upon surfaces, which is rich in bacteria, and when dried this material is easily carried into the atmosphere by currents of air.

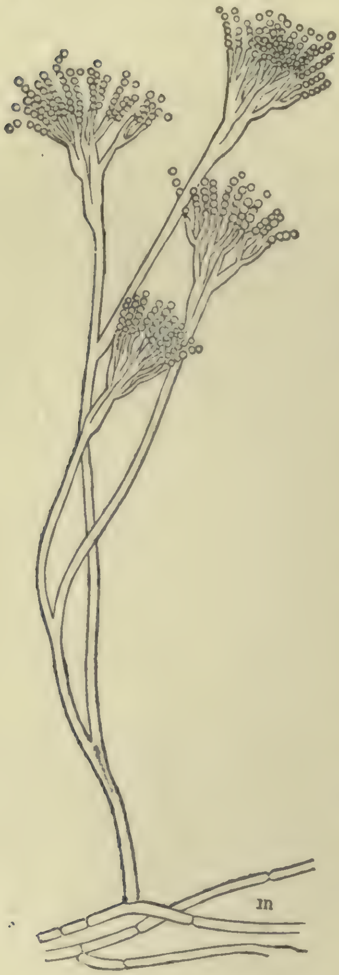


FIG. 186.—**Penicillium glaucum; *m*, mycelium, from which is given off a branching pedicle bearing spores. × 150.**

Tyndall's experiments (1869) show that in a closed receptacle in which the air is perfectly still all suspended particles are after a time deposited on the floor of the closed air chamber. And common experience demonstrates the fact that the dust of the atmosphere is carried by the wind from exposed surfaces and again deposited when the air is at rest. This dust as deposited, for example, in our dwellings contains innumerable bacteria in a desiccated condition, and the smallest quantity of it introduced into a sterile organic liquid will cause it to undergo putrefactive decomposition, and by bacteriological methods it will be found to contain various species of bacteria. Such dust also contains the spores of various mould fungi which are present in the atmosphere, usually in greater numbers than the bacteria. The mould fungi are air plants which vegetate upon the surface of moist organic material and form innumerable spores, which are easily wafted into the air, both on account of their low specific gravity and minute size, and because they are borne upon projecting pedicles by which they are removed from the moist material upon which and in which the mycelium develops (Fig. 186), and, being dry, are easily carried away by currents of air.

Bacteriologists have given much attention to the study of the microörganisms suspended in the atmosphere, with especial reference to hygienic questions. The methods and results of these investigations will be considered in the present section.

Pasteur (1860) demonstrated the presence of living bacteria in the atmosphere by aspirating a considerable quantity of air through a filter of gun-cotton or of asbestos contained in a glass tube. By dissolving the gun-cotton in alcohol and ether he was able to demonstrate the presence of various microörganisms by a microscopical examination of the sediment, and by placing the asbestos filters in sterilized culture media he proved that living germs had been filtered out of the air passed through them.

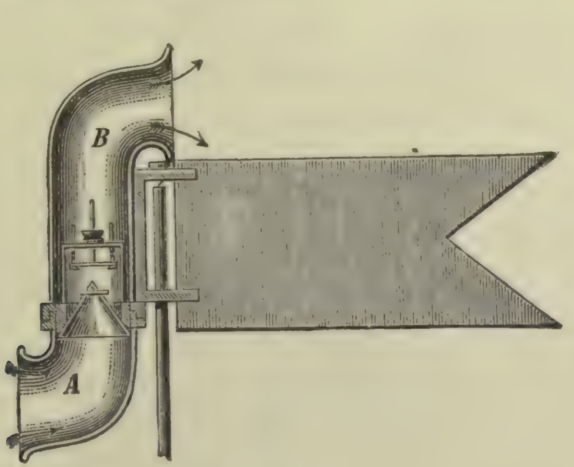


FIG. 187.

A method employed by several of the earlier investigators consisted in the collection of atmospheric moisture precipitated as dew upon a surface cooled by a freezing mixture. This was found to contain living bacteria of various forms. The examination of rain water, which in falling washes the suspended particles from the atmosphere, gave similar results.

The first systematic attempts to study the microörganisms of the air were made by Maddox (1870) and by Cunningham (1873), who used an aëroscope which was a modification of one previously described by Pouchet. In the earlier researches of Miquel a similar aëroscope was used. This is shown in Fig. 187. The opening to the cylindrical tube A is kept facing the wind by means of a wind vane, and when the wind is blowing a current passes through a small aperture in a funnel-shaped partition which is properly placed in the cylindrical tube. A glass slide, upon the lower surface of which a mixture of glycerin and glucose has been placed, is adjusted near the opening of the funnel, at a distance of about three millimetres, so that the air escaping through the small orifice is projected against it. By this arrangement a considerable number of the microörganisms present in the air, as well as suspended particles of all kinds, are arrested upon the surface of the slide and can be examined under the microscope or studied by bacteriological methods. But an aëroscope of this kind gives no precise information as to the number of living germs contained in a definite quantity of air. The microscopical examination also fails to differentiate the bacteria from particles of various kinds which resemble them in shape, and the microörganisms seen are for the most part spores of various fungi mingled with pollen grains, vegetable fibres, plant hairs, starch granules, and amorphous granular material.

Another method, which has been employed by Cohn, Pasteur, Miquel, and others, consists in the aspiration of a definite quantity of air through a culture liquid, which is then placed in an incubating oven for the development of microörganisms washed out of the air which has been passed through it. This method shows that bacteria of different species are present, but gives no information as to their relative number, and requires further researches by the plate method to determine the characters of the several species in pure cultures.

A far simpler method consists in the exposure of a solid culture medium, which has been carefully sterilized and allowed to cool on a glass plate or in a Petri's dish, for a short time in the air to be examined. Bacteria and mould fungi deposited from the air adhere to the surface of the moist culture medium, and form colonies when the plate, enclosed in a covered glass dish, is placed in the incubating oven. The number of these colonies which develop after exposure in the air for a given time enables us to estimate in a rough way the number of microörganisms present in the air of the locality where the exposure was made ; and the variety of species is determined by examining the separate colonies,each of which is, as a rule, developed from a single germ. By exposing a number of plates at different times this method enables us to determine what species are most abundant in a given locality and the comparative number in different localities, as determined by counting the colonies after exposure for a definite time—*e.g.*, ten minutes. Of course we will only obtain evidence of the presence of such aërobic bacteria as will grow in our culture medium. The anaërobic bacteria may be studied by placing plates exposed in a similar way in an atmosphere of hydrogen. Bacteria which grow slowly and only under special conditions, like the tubercle bacillus, would be likely to escape observation, as the mould fungi and common saprophytes would take complete possession of the surface of the culture medium before the others had formed visible colonies. Students will do well to employ this simple and satisfactory method for the purpose of making themselves familiar with the more common atmospheric organisms, and they will find the shallow glass dishes with a cover, known as Petri's dishes, very convenient for the purpose. These dishes should be sterilized in the hot-air oven and sufficient sterile nutrient gelatin or agar poured into them to cover the bottom. After the culture medium has become solid by cooling, the exposure may be made by simply removing the cover and replacing it at the end of the time fixed upon.

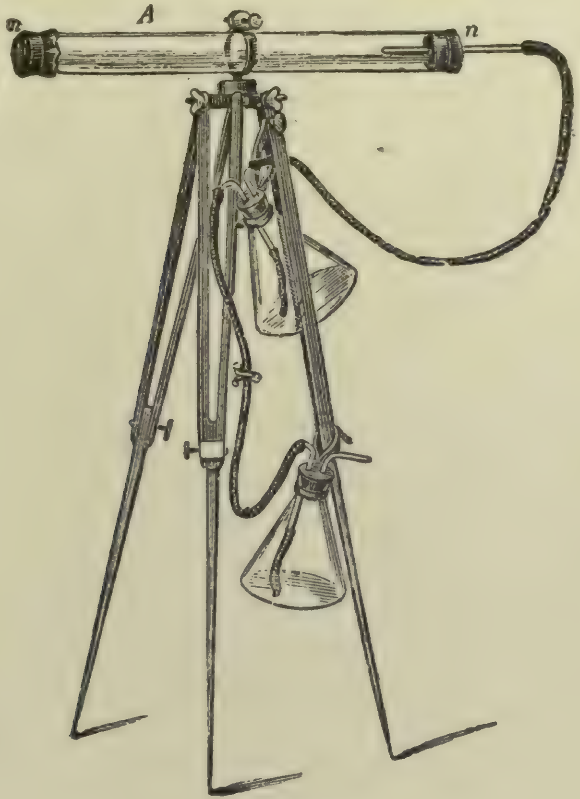


FIG. 188.

To determine in a more exact way the number of microörganisms contained in a given quantity of air will require other methods. But we may say, *en passant*, that such a determination is usually not of great scientific importance. The number is subject to constant fluctuations in the same locality, depending upon the force and direction of the wind. If we have on one side of our laboratory a dusty street and on the other a green field, more bacteria will naturally be found when the wind blows from the direction of the street than when it comes from the opposite direction ; or, if the air is filled with dust from recently sweeping the room, we may expect to find very many more than when the room has been undisturbed for some time. The painstaking researches which have already been made have established in a general way the most important facts relating to the distribution of atmospheric bacteria, but have failed to show any definite relation between the number of atmospheric bacteria and the prevalence of epidemic diseases. In the apparatus of Hesse, Fig. 188, a glass tube, having a diameter of four to five centimetres and a length of half a metre to a metre, is employed. In use this is supported upon a tripod, as shown in the figure, and air is drawn through it by a water aspirator consisting of two flasks, also shown. The upper flask being filled with water, this flows into the lower flask by siphon action, and upon reversing the position of the flasks number one is again filled. By repeating this operation as many times as desired a quantity of air corresponding with the amount of water passed from the upper to the lower flask is slowly aspirated through the horizontal glass tube. The microörganisms present are deposited upon nutrient gelatin previously allowed to cool upon the lower portion of the large glass tube. The air enters through a small opening in a piece of sheet rubber which is tied over the extremity of the horizontal tube, and before the aspiration is commenced this opening is covered by another piece of sheet rubber tied over the first. Experience shows that when the air is slowly aspirated most of the germs contained in it are deposited near the end of the tube through which it enters. The colonies which develop upon the nutrient gelatin show the number and character of living microörganisms contained in the measured quantity of air aspirated through the apparatus. The method with a soluble filter of pulverized sugar, to be described hereafter, is preferable when exact results are desired; and for the purpose of determining the relative abundance and the variety of microörganisms present in the atmosphere of a given locality the exposure of nutrient gelatin in Petri's dishes is far simpler, and, as a rule, will furnish all the information that is of real value.

In his extended researches made at the laboratory of Montsouri, in Paris, Miquel has used various forms of apparatus and has obtained interesting results; but his method of *ensemencements fractionnés* requires a great expenditure of time and patience, and the more recent method with soluble filters is to be preferred.

In his latest modification of the method referred to Miquel used a flask like that shown in Fig. 189. From twenty to forty cubic centimetres of distilled water are introduced into this flask. The cap A contains a cotton air filter and is fitted to the neck of the flask by a ground joint. This is removed during the experiment. The tube C is connected with an aspirator. It contains two cotton or asbestos filters, *c* and *b*. The cap being removed and the aspirator attached, the air is drawn through the water, by which suspended germs are arrested; or if not they are caught by the inner cotton plug *b*. The sealed point of the tube B is now broken off, and the contents of the flask equally divided in thirty to forty tubes containing bouillon, which are placed in the incubating oven. Twenty-five cubic centimetres of bouillon are also introduced into the flask, and the cotton plug *b* is pushed into it so that any bacteria arrested by it may develop. If one-fourth or one-fifth of the bouillon tubes show a development of bacteria it is inferred that each culture originated from a single germ, and the number present in the amount of air drawn through the flask is estimated from the number of tubes in which development occurs.

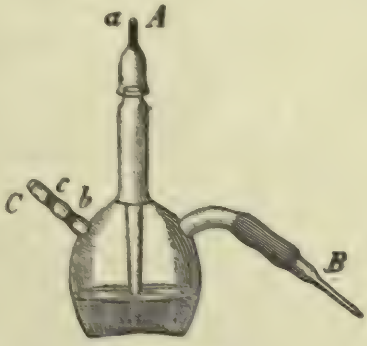


FIG. 189.

The method adopted by Straus and Würtz is more convenient and more reliable in its results. This consists in passing the air by means of an aspirator through liquefied nutrient gelatin or agar. The apparatus shown in Fig. 190 is used for this purpose. Two cotton plugs are placed in the tube B, to which the aspirator is attached, and after the determined quantity of air has been passed through the liquefied medium the inner plug is pushed down with a sterilized platinum needle so as to wash out in the culture medium any germs arrested by it. Finally the gelatin or agar is solidified upon the walls of the tube A by rotating it upon a block of ice or under a stream of cold water. It is now put aside for the development of colonies, which are counted to determine the number of germs present in the quantity of air passed through the liquefied culture medium. The main difficulty with this apparatus is found in the fact that the nutrient gelatin foams when air is bubbled through it; for this reason an agar medium is to be preferred. In using this it will be necessary to place the liquefied agar in a bath maintained at 40° C. Foaming of the gelatin is prevented by adding a drop of olive oil before sterilization in the steam sterilizer. But this interferes with the transparency of the medium.

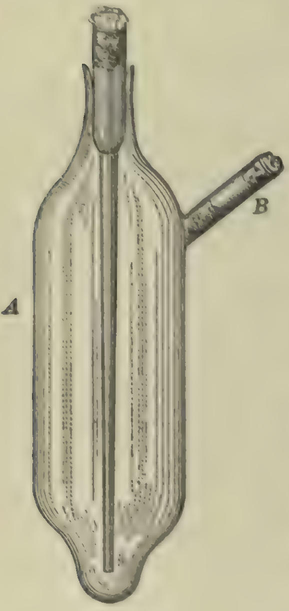


FIG. 190.

In the earlier experiments upon atmospheric organisms Pasteur used a filter of asbestos, which was subsequently washed out in a culture liquid. A filter of this kind washed out in liquefied gelatin or nutrient agar would give more satisfactory results, as the culture medium could be poured upon plates or spread upon the walls of a test tube and the colonies counted in the usual way. Petri prefers to use a filter of sand, which he finds by experiment arrests the microörganisms suspended in the atmosphere, and which is subsequently distributed through the culture medium. The sand used is such as has been passed through a wire sieve having openings of 0.5 millimetre in diameter. This is sterilized by heat, and is supported in a cylindrical glass tube by small wire-net baskets. The complete arrangement is shown in Fig. 191. Two sand filters, c_1 and c_2, are used, the lower one of which serves as a control to prove that all microörganisms present in the air have been arrested by the upper one. The upper filter is protected, until the aspirator attached to the tube *h* is put in operation, by a sterile cotton plug, not shown in the figure which represents the filter in use. Petri uses a hand air pump as an aspirator, and passes one hundred litres of air through the sand in from ten to twenty minutes. The sand from the two filters is then distributed in shallow glass dishes and liquefied gelatin is poured over it; this is allowed to solidify and is put aside for the development of colonies. The principal objection to this method is the presence of the opaque particles of sand in the culture medium. This objection has been overcome by the use of soluble filters, a method first employed by Pasteur and since perfected by Sedgwick and by Miquel. The most useful material for the purpose appears to be cane sugar, which can be sterilized in the hot-air oven at 150° C. without undergoing any change in its physical characters. Loaf sugar is pulverized in a mortar and passed through two sieves in order to remove the coarser grains and the very fine powder, leaving for use a powder having grains of about one-half millimetre in diameter. This powdered sugar is placed in a glass tube provided with a cap having a ground joint and a cotton plug to serve as an air filter (A, Fig. 192), or in a tube such as is shown at B, having the end drawn out and hermetically sealed. Two cotton plugs are placed at the lower portion of the tube, at *a* and at *b*.

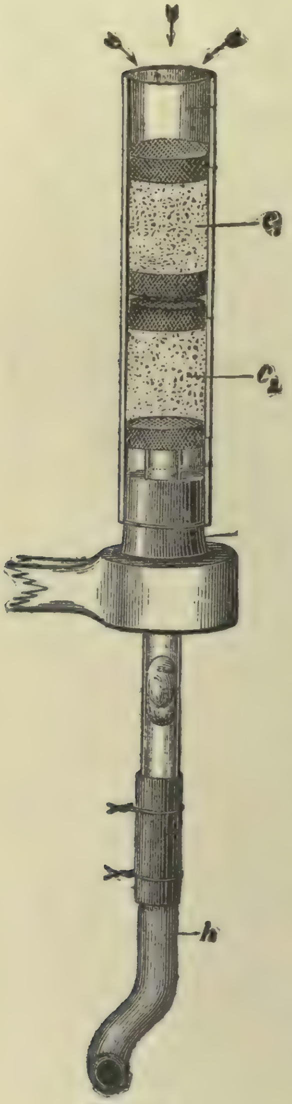


Fig. 191.

Glass tubing having a diameter of about five millimetres is used in making these tubes, and from one to two grammes of powdered sugar is a suitable quantity to use as a filter. The whole apparatus is sterilized for an hour at 150° C. in a hot-air oven after the pulverized sugar has been introduced. Before using it will be necessary to pack the sugar against the supporting plug *a* by gently striking the lower end of the tube, held in a vertical position, upon some horizontal surface; and during aspiration the tube must remain in a vertical position, or nearly so, in order that the sugar may properly fill its entire calibre. The aspirator is attached to the lower end of the tube by a piece of rubber tubing. When the tube B is used the sealed extremity is broken off at the moment that the aspirator is set in action, and it is again sealed in a flame after the desired amount of air has been passed through the filter. The next step consists in dissolving the sugar in distilled water or in liquefied gelatin. To insure the removal of all the sugar the cotton plug *a* may be pushed out with a sterilized glass rod, after removing *b* with forceps. From fifty to five hundred cubic centimetres of distilled water, contained in an Erlenmeyer flask and carefully sterilized, may be used, the amount required depending upon circumstances relating to the conditions of the experiment. By adding five or ten cubic centimetres of this water, containing the sugar and microörganisms arrested by it, to nutrient gelatin or agar liquefied by heat, and then making Esmarch roll tubes, the number of germs in the entire quantity is easily estimated by counting the colonies which develop in the roll tubes.

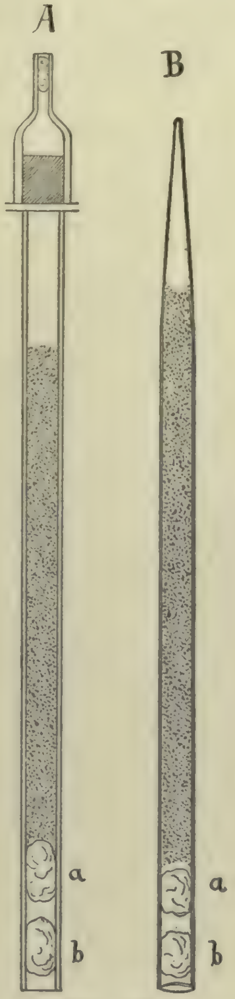


FIG. 192.

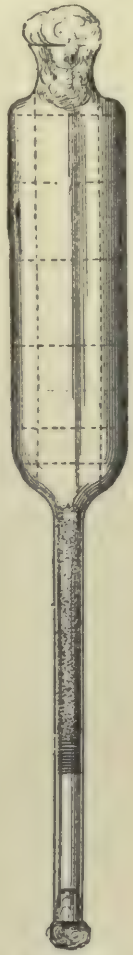


FIG. 193.

Sedgwick and Tucker, in a communication made to the Boston Society of Arts, January 12th, 1888, were the first to propose the use of a soluble filter of granulated sugar for collecting atmospheric germs. Their complete apparatus consists of an exhausted receiver, from which a given quantity of air is withdrawn by means of an air pump. A vacuum gauge is attached to the receiver, which is coupled with the glass tube containing the granulated-sugar filter by a piece of rubber tubing. Instead of transferring the soluble filter to gelatin in test tubes, they use a large glass cylinder having a slender stem, in which the sugar is placed (Fig. 193). After the aspiration liquefied gelatin is introduced into the large glass cylinder, which is held in a horizontal position ; the sterilized cotton plug is then replaced in the mouth of the cylinder, the sugar is pushed into the liquefied gelatin and dissolved, and by rotating the cylinder upon a block of ice the gelatin is spread upon its walls as in an Esmarch roll tube. For convenience in counting the colonies lines are drawn upon the surface of the cylinder, dividing it into squares of uniform dimensions.

GENERAL RESULTS OF RESEARCHES MADE.

As already stated, the presence of bacteria in the atmosphere depends upon their being wafted by currents of air from surfaces where they are present in a desiccated condition. That they are not carried away from moist surfaces is shown by the fact that expired air from the human lungs does not contain microörganisms, although the inspired air may have contained considerable numbers, and there are always a vast number present in the salivary secretions. The moist mucous membrane of the respiratory passages constitutes a germ trap which is much more efficient than the glass slide smeared with glycerin used in some of the aëroscopes heretofore described, for it is a far more extended surface. As a matter of fact, most of the suspended particles in inspired air are deposited before the current of air passes through the larynx.

Air which passes over large bodies of water is also purified of its germs and other suspended particles. The researches of Fischer show that at a considerable distance from the land no germs are found in the atmosphere over the ocean, and that it is only upon approaching land that their presence is manifested by the development of colonies upon properly exposed gelatin plates.

Uffelmann found, in his researches, that in the open fields the number of living germs in a cubic metre of air averaged two hundred and fifty, on the sea coast the average was one hundred, in the courtyard of the University of Rostock four hundred and fifty. The number was materiallv reduced after a rainfall and increased when a dry land wind prevailed.

Frankland found that fewer germs were present in the air in winter than in summer, and that when the earth was covered with snow the number was greatly reduced, as also during a light fall of snow ; the air of towns was found to be more rich in germs than the air of the country; the lower strata of the atmosphere contained more than the air of elevated localities.

Von Freudenreich also found that the air of the country contained fewer germs than that of the city. Thus in the city of Berne a cubic metre of air often contained as many as two thousand four hundred germs, while the maximum in country air was three hundred. His results corresponded with those of Miquel in showing that the number of atmospheric organisms is greater in the morning and the evening, between the hours of 6 and 8, than during the rest of the day. Neumann, whose researches were made in the Moabite Hospital, found the greatest number of bacteria in the air in the morning after the patients able to sit up had left their beds and the wards had been swept. The number of germs was then from eighty to one hundred and forty in ten litres of air, while in the evening the number fell to four to ten germs in ten litres.

Miquel has given the following summary of results obtained in his extended experiments, made in Paris during the years 1881, 1882, and 1883:

	Number of Germs in a Cubic Metre of Air.	
	Air of Laboratory, Montsouri.	Air of Park, Mont souri.
Average for 1880	215	71
" " 1881	348	62
" " 1882	550	51

Rue de Rivoli, average for one year, 750; summit of Pantheon, 28; Hôtel-Dieu, 1880, average for four months, male ward 6,300, female ward 5,120; La Piété Hospital, average of fifteen months, 11,100.

It must be remembered that the figures given relate both to bacteria and to the spores of mould fungi, and that the latter are commonly the most numerous when the experiment is made in the open air. Petri has shown that when gelatin plates are exposed in the air the relative number of spores of mould fungi deposited upon them is less than is obtained in aspiration experiments.

The number of colonies which develop on exposed plates does not represent the full number of bacteria deposited, for these colonies very frequently have their origin in a dust particle to which several bacteria are attached, or in a little mass of organic material containing a considerable number.

It is generally conceded that sea air and country air are more wholesome than the air of cities, and especially of crowded apartments, in which the number of bacteria has been shown to be very much greater. But it would be a mistake to ascribe the sanitary value of sea, country, and mountain air to the relatively small number of bacteria present in such air. There are other important factors to be considered, and we have no satisfactory evidence that the number of saprophytic bacteria present in the air has an important bearing upon the health of those who respire it. We do know that the confined air of crowded apartments, and especially of factories in which a large quantity of dust is suspended in the air, predisposes those breathing such air to pulmonary diseases and lowers the general standard of health. But it has not been proved that this is due to the presence of bacteria. Infectious diseases may, under certain circumstances, be communicated by way of the respiratory passages as a result of breathing air containing in suspension pathogenic bacteria ; but there is reason to believe that this occurs less frequently than is generally supposed.

Krüger has shown that the dust of a hospital ward in which patients with pulmonary consumption expectorated occasionally upon the floor contained tubercle bacilli. This was proved by wiping up the dust on a sterilized sponge, washing this out in bouillon, and injecting this into the cavity of the abdomen of guinea-pigs. Two animals out of sixteen injected became tuberculous. In pulmonic anthrax, which occasionally occurs in persons engaged in sorting wool—" wool-sorters' disease"—infection occurs as a result of the respiration of air containing the spores of the anthrax bacillus.

Among the non-pathogenic saprophytes found in the air certain aërobic micrococci appear to be the most abundant, and, as a rule, bacilli are not found in great numbers or variety. In some localities various species of sarcinæ are especially abundant. The following is a partial list of the species which have been shown by the researches of various bacteriologists to be occasionally present in the air. But, as heretofore remarked, their presence is to be regarded as accidental, and so far as we know there is no bacterial flora properly belonging to the atmosphere :

Micrococcus ureæ (Pasteur), Diplococcus roseus (Bumm), Diplococcus citreus conglomeratus (Bumm), Micrococcus radiatus (Flügge), Micrococcus flavus desidens (Flügge), Micrococcus flavus liquefaciens (Flügge), Micrococcus tetragenus versatilis (Sternberg), Micrococcus pyogenes aureus (Rosenbach), Micrococcus pyogenes citreus (Passet), Micrococcus cinnabareus (Flügge), Micrococcus flavus tardigradus (Flügge), Micrococcus versicolor (Flügge), Micrococcus viticulosus (Katz), Micrococcus candidans (Flügge), Pediococcus cerevisiæ (Balcke), Sarcina lutea (Schröter), Sarcina rosea (Schröter), Sarcina aurantiaca, Sarcina alba, Sarcina candida (Reinke), Bacillus tumescens (Zopf), Bacillus subtilis (Ehrenberg), Bacillus multipediculosus (Flügge), Bacillus mesentericus fuscus (Flügge), Bacillus mesentericus ruber (Globig), Bacillus inflatus (A. Koch), Bacillus mesentericus vulgatus, Bacillus prodigiosus, Bacillus aërophilus (Liborius), Bacillus pestifer (Frankland), Spirillum aureum (Weibel), Spirillum flavescens (Weibel), Spirillum flavum (Weibel), Bacillus Havaniensis (Sternberg).

In the researches of Welz, made in the vicinity of Freiburg, twenty-three different micrococci and twenty-two bacilli were obtained from the air.

ADDITIONAL NOTES UPON BACTERIA IN THE AIR.

Ruete and Enoch (1895) have examined the air of closed schoolrooms with the following results. Eighteen different species were obtained, only one of which proved to be pathogenic for mice, guinea-pigs, and rabbits. The number of bacteria per cubic metre varied from 1,500 to 3,000,000, the average being about 268,000. The observations were made during the winter months.

Marpmann (1893), in his examination of dust collected in the streets of Leipzig for tubercle bacilli, obtained positive results from a considerable proportion of the specimens examined. Evidently these bacilli in dust from the streets are liable to be blown into the air and deposited upon the mucous membrane of the respiratory passages of those breathing this air. Christiani (1893) has shown that, as a rule, no bacteria are present in the air at an altitude of one thousand metres or more above the soil (air collected during balloon ascensions).

Dyar (1895) has made a careful study of the microörganisms found in the air in the city of New York. He has described numerous species of micrococci and bacilli found chiefly in the air of the hallway of the College of Physicians and Surgeons. Some of these are new and some have been identified as previously described species.

II.

BACTERIA IN WATER.

THE water of the ocean, of lakes, ponds, and running streams necessarily contains bacteria, as they are constantly being carried into it by currents of air passing over the neighboring land surfaces, and by rain water which washes suspended microörganisms from the atmosphere ; and, as such water contains more or less organic material in solution, many of the saprophytic bacteria multiply in it abundantly. It is only in the water of springs and wells which comes from the deeper strata of the soil that they are absent. The number and variety of species present in water from any given source will depend upon conditions relating to the amount of organic pabulum, the temperature, the depth of the water, the fact of its being in motion or at rest, its pollution from various sources, etc. The comparatively pure water of lakes and running streams contains a considerable number of bacteria which find their normal habitat in such waters and which multiply abundantly in them, notwithstanding the small quantity of organic matter and salts which they contain. The water of stagnant, shallow pools, and of sluggish streams into which sewage is discharged, contains a far greater number and a greater variety of species.

The study of these bacteria in water has received much attention on account of the sanitary questions involved, relating to the use of water from various sources for drinking purposes. In the present section we shall first give an account of the methods of bacteriological water analysis, and then a condensed statement of results obtained in the very numerous investigations which have been made.

A very important point to be kept in view is the fact that a great increase in the number of bacteria present, in samples of water collected for investigation, is likely to occur if these samples are kept for some time. A water which, for example, contains only two hundred to three hundred bacteria per cubic centimetre when the examination is made at once, may contain several thousand at the end of twenty-four hours, and at the end of the second or third day twenty thousand or more may be present in the same quantity.

Later, on account of the exhaustion of organic pabulum, the number is again reduced as the bacteria present gradually lose their vitality. Under these circumstances it is evident that an estimate of the number of bacteria present in water from a given source can have no value, unless a sample is tested by bacteriological methods within a short time after it has been collected. Not more than an hour or two should be allowed to elapse, especially in warm weather. By placing the water upon ice the time may be extended somewhat, but Wolffhügel has shown that the number of germs is gradually diminished when water is preserved in this way, and it will be safest to make an immediate examination when this is practicable.

The *collection* may be made in a sterilized Erlenmeyer flask provided with a cotton air filter, or in a bottle having a ground-glass stopper which has been wrapped in tissue paper and sterilized for an hour or more at 150° C. in the hot-air oven. Or the small flasks with a long neck may be used, as first recommended by Pasteur. These are prepared as follows: The bulb is first gently heated, and the extremity of the tube dipped into distilled water, which mounts into the bulb as it cools; the water is then made to boil, and when all but a drop or two has escaped and the bulb is filled with steam the extremity of the tube is hermetically sealed. When the steam has condensed by the cooling of the bulb a partial vacuum is formed, and the tube is ready for use at any time. It is filled with water by breaking off the sealed extremity under the surface of the water of which a sample is desired. This is done with sterilized forceps, and care must be taken that the exterior of the tube is properly sterilized before the collection is made. The end is immediately sealed in the flame of a lamp. A difficulty with these vacuum tubes is that they are so completely filled with water that this cannot be readily drawn from them again in small quantities. The writer therefore prefers to make the collection in a tube shaped as shown in Fig. 194, in which a partial vacuum is formed just before the collection by heating the air in the bulb. The water mounts into the tube as the air in the bulb cools, and is readily forced out again for making cultures by applying gentle heat to the bulb. As a lamp is needed to seal the end of the tube in either case, there is no special advantage in having a vacuum formed in advance, and, as stated, the vacuum tubes are so

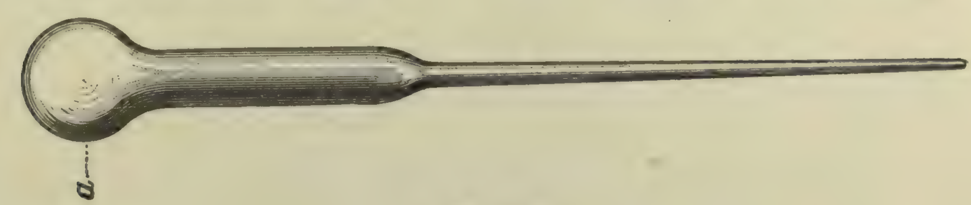


FIG. 194.

nearly filled with water that it is not so simple a matter to obtain the contents for our culture experiments without undue exposure to atmospheric germs. In practice small glass bottles with ground-glass stoppers will be found most convenient, and, when properly sterilized, are unobjectionable. They should be filled at a little distance below the surface, as there is often a deposit of dust upon the surface of standing water, and sometimes a delicate film made up of aërobic bacteria. When water is to be obtained from a pump or a hydrant it should be allowed to flow for some time before the collection is made. To collect water at various depths the apparatus shown in Fig. 195 is recommended by Lepsius. An iron frame supports an inverted flask, A, filled with sterilized mercury and containing about three hundred cubic centimetres. The flask B is intended to receive the mercury when, at the desired depth, it is allowed to flow through the capillary tube *b*. This is sealed at the extremity and bent as shown in the figure. By pulling upon the cord *c* this tube is broken, and as the mercury flows from the flask this is filled with water through the tube *a*. The extremity of the broken tube *b* is closed by the mercury in the flask B when A is full of water, and the apparatus can be brought to the surface with only such water as was collected at the depth from which a sample was desired.

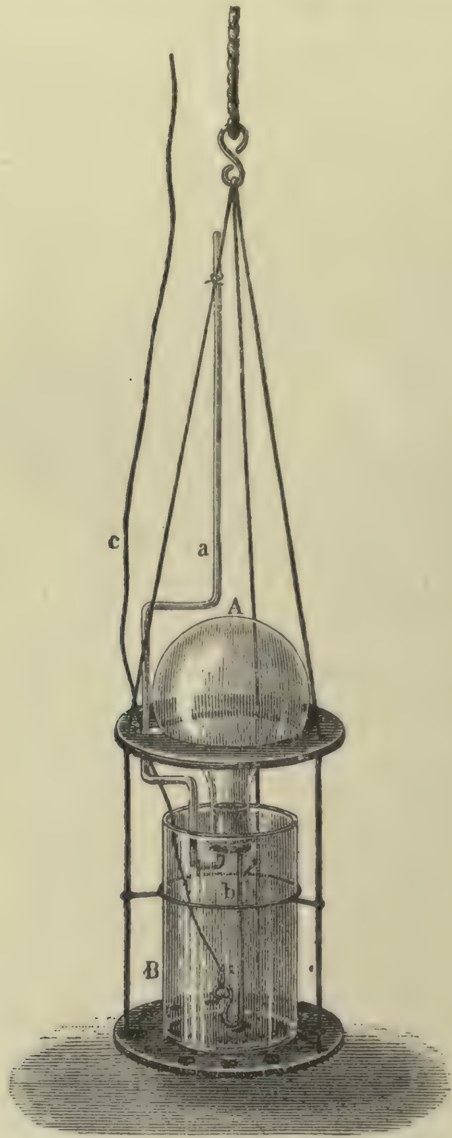


FIG. 195.

The *bacteriological analysis* is made by adding a definite quantity of the water under investigation to liquefied gelatin or agar-gelatin, and making a plate or Esmarch roll tube, which is put aside for the development of colonies. Miquel and others have preferred to use liquid cultures and the method of fractional cultivation described in the previous section. The use of a solid culture medium has, however, such obvious advantages that we do not consider it necessary to do more than refer to the other method as one which, when applied with skill and patience, may give sufficiently accurate results.

The amount of water which should be added to the usual quantity of liquefied flesh-peptone-gelatin in a test tube, in order that the colonies which develop may be well separated from each other and easily counted, can only be determined by experiment. If the water is from an impure source a single drop may be too much, and it will be necessary to dilute it with distilled water recently sterilized. But for ordinary potable water it will usually be best, in a first experiment, to make two trials, one with one cubic centimetre and one with one-half cubic centimetre added to the liquefied nutrient gelatin. The water in the collecting bottle should be shaken, to distribute the bacteria which may have settled to the bottom, before drawing off by means of a sterilized pipette the amount used for the experiment, and the germs present in it are to be distributed through the liquefied gelatin by gently moving the tube to and fro.

Koch's method of preparing a gelatin plate is illustrated in Fig. 196. A glass dish, containing ice water and covered with a large plate of glass, is supported upon a levelling tripod. By means of a spirit level this is adjusted to a horizontal position, so that when the liquefied gelatin is poured upon the smaller sterilized glass plate, seen in the centre of the large plate of glass, it will not flow, but may be evenly distributed over the surface by means of a sterilized glass rod. The glass cover resting against the side of the apparatus is placed over the gelatin plate while it is cooling, to protect it from atmospheric germs, and when the gelatin is hard the plate is transferred to a shallow glass dish, which is kept at a temperature of about 20° C. for several days for the development of colonies. It is difficult to count colonies when more than five thousand develop upon a plate of the usual size, and for this reason it will be best to repeat the experiment with a smaller quantity of water from the same source, if this is at hand, rather than to attempt to count an overcrowded plate. Before pouring the gelatin upon the plate the lip of the test tube containing it should be sterilized by passing it through a flame. The liquefied gelatin should be carefully distributed to cover a rect-

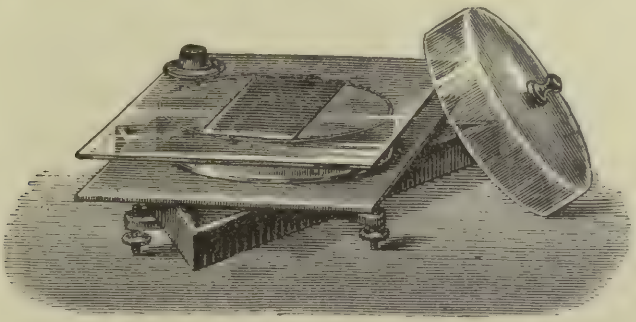


FIG. 196.

angular surface and leaving a margin of about one centimetre around the edge of the plate. The Koch's dish in which the gelatin plate is placed for the development of colonies should be carefully sterilized by heat or by washing it out with a sublimate solution. A circular piece of filtering paper, saturated with sublimate solution or distilled water, is placed at the bottom of the lower dish to keep the air in a moist condition and prevent drying of the gelatin. Usually two or three plates made at the same time are placed one above the other on glass supports made for this purpose. If many liquefying organisms are present it will be necessary to count the colonies before these run together—usually on the second day; but in the absence of liquefying colonies it is best to wait until the third, or even the fifth day, as the number of visible colonies and the ease of counting them will be greater than at an earlier date. The development of a few scattered liquefying colonies which threaten to spoil the plate may be arrested by taking up the liquefied gelatin from each with a bit of filtering paper, and then, by means of a camel's-hair brush, applying a solution of potassium permanganate to the margin of the colony. The growth of colonies of mould fungi, which have developed from spores from the atmosphere falling upon the plate while it is exposed, can be checked by the application of collodion containing bichloride of mercury.

Counting of the colonies is a simple matter when they are few in number; when they are numerous it is customary to place the plate over a dark background, and to place above it a glass plate divided into square centimetres by lines ruled with a diamond. By means of a lens of low power the colonies in a certain number of squares are counted and the average taken. This multiplied by the number of square centimetres in the gelatin-covered surface gives approximately the entire number of colonies which have developed from the amount of water used in the experiment.

Instead of using Koch's original plate method, as above described, the shallow, covered glass dishes recommended by Petri may be employed. These are from one to one and one-half centimetres high and from ten to fifteen centimetres in diameter. The liquefied gelatin is poured into the lower dish and the cover at once placed over it. The gelatin does not dry out very soon, but, if necessary, several of these Petri's dishes may be placed in a larger jar, which serves as a moist chamber.

The roll tubes of Esmarch may also be used, and have the advantage that accidental colonies from air-borne germs are excluded. The counting of colonies is not quite as easy, but by the use of a mounted lens especially designed for the purpose it is attended with no great difficulty. The surface of the tube is divided into squares by colored lines, and the number of colonies in several squares is counted in order to obtain an average and estimate the entire number.

Water which contains numerous liquefying bacteria had better be examined by the use of nutrient agar instead of gelatin; and in very warm weather it will be necessary to use an agar medium, as ten-per-cent gelatin is likely to melt if the temperature goes above 22° C. A difficulty in the use of agar for plates consists in the liability of the film to slip from the glass. This may be remedied to some extent by adding a few drops of a concentrated solution of gum acacia to the liquefied agar medium. Petri's dishes are well adapted for the use of the agar medium, as the objection referred to does not apply to them. The gelatin-agar medium, containing 5 per cent of gelatin and 0.75 per cent of agar, may also be used with advantage in the bacteriological analysis of water. Much stress was at one time laid upon the enumeration of liquefying colonies, upon the supposition that the liquefying bacteria were especially harmful as compared with the non-liquefying, and that a water containing many liquefying colonies was to be looked upon with suspicion. We now know, however, that there are many common and harmless saprophytes which cause the liquefaction of gelatin, and that some of the most dangerous pathogenic bacteria do not liquefy gelatin. This distinction has therefore no special value, and the question for bacteriologists to-day is not how large is the comparative number of liquefying colonies, but what species are represented by the colonies present, liquefying and non-liquefying, and what are the special pathogenic properties of each. The answer to these questions, in the case of any particular water supply, calls for special knowledge and great patience and care in the isolation in pure cultures, and careful study of the various species present.

It is now generally recognized that a mere enumeration of the number of colonies which develop from a water under investigation is not a sufficient indication upon which to found an opinion as to its potability. An excessive number of bacteria is an indication that the water contains a large amount of the organic material which serves as pabulum for these microörganisms. But the chemists are able to determine the amount of organic matter present in water with greater precision; and, as we have seen, the number of bacteria may increase many-fold in water which is kept standing in the laboratory for two or three days in a well-corked bottle. As a matter of fact, the enumeration of bacteria in water, although it has given us results of scientific interest, has not materially added to the methods previously applied for estimating the sanitary value of water obtained from various sources for drinking purposes. But the bacteriological examination may prove to be of great value if it succeeds in demonstrating the presence of certain pathogenic bacteria and in thus preventing the use of a dangerous water. We do not mean to say, however, that an enumeration of the bacteria present in drinking water has no practical value. An excessive number indicates an excessive amount of organic pabulum, which may have come from a dangerous source; and the dangerous pathogenic bacteria are not only more likely to be present in such water, but they can more readily multiply in it, while in a pure water they would fail to increase in number, and, as has been shown by experiment, would die out within a short time.

The number of bacteria present in *rain water*, or in *snow* which has recently fallen, varies greatly at different times. Naturally the number is greater when the surface of the earth is dry and the atmosphere loaded with dust by currents of wind passing over it, and less when the surface is moist and the atmosphere has been purified by recent rains.

In snow from the surface of a glacier in Norway, Schmelck found two bacteria and two spores of mould fungi per cubic centimetre of water from the melted snow. Ganowski, in experiments made with freshly fallen snow collected in the vicinity of Kiew, obtained the following results: February 2d, 1888: temperature of the air, 7.2° C.; snowfall, 0.1 millimetre; number of bacteria in 1 cubic centimetre of water from melted snow, 34 in one sample and 38 in another. February 20th, 1888: temperature, 11.1° C.; snowfall, 1.1 millimetres; number of bacteria in one sample, 203, in another 384.

Miquel obtained from rain water collected at Montsouri during a rainy season 4.3 germs per cubic centimetre; in rain water collected in the centre of the city of Paris, 19 per cubic centimetre.

Hail has also been shown to contain bacteria in considerable numbers. Bujwid found in hailstones which fell at Warsaw 21,000 bacteria in 1 cubic centimetre; but this is exceptional, and is supposed to be due to the fact that surface water had been carried into the air by the storm and frozen. Fontin examined hail which fell in St. Petersburg, and obtained an average of 729 bacteria per cubic centimetre of water from the melted hail.

River water has been carefully examined by numerous bacteriologists in various localities and at different seasons of the year. We give below some of the results reported:

Water of the Seine at Choisy, before reaching Paris, 300; at Bercy, 1,200; at Saint-Denis, after receiving the sewer water from the city, 200,000 germs per cubic centimetre (Miquel).

Water of the Spree beyond Köpenick, 82,000; two hundred steps below the mouth of the Wuhle, 118,000; in Berlin above the mouth of the Panke, 940,000; below the mouth of the Panke, 1,800,000 (Koch).

Water of the Main above the city of Würzburg, in the month of February, 520; below the city, 15,500 (Rosenberg).

Water of the Potomac, at Washington, in 1886: January, 3,774; February, 2,536; March, 1,210; April, 1,521; May, 1,064; June, 348; July, 255; August, 254; September, 178; October, 75; November, 116; December, 967 (Theobald Smith).

The Thames, in the autumn of 1885, in the vicinity of London Bridge two hours after high water, contained 45,000 germs per cubic centimetre; the water of the Lea at Lea Bridge, 4,200,000 (Bischoff).

The Neva inside the city of St. Petersburg, in September, 1883, contained 1,500 in one sample and 1,040 in another; in November (20th), 6,500 (Poehl).

The water of the Oder, collected within the limits of the city of Stettin, was found by Link to contain from 5,240 to 15,000 bacteria per cubic centimetre; that of the Limmat, at Zurich, 346 in one specimen and 508 in another (Cramer).

Lake water, as a rule, contains fewer bacteria than river water.

Wolffhügel, in researches extending from July, 1884, to July, 1885, obtained from the water of the Tegeler Lake an average of 396 bacteria per cubic centimetre. Cramer obtained an average of 168 per cubic centimetre during the months of October, December, and January, 1884, from the water of Lake Zurich; in June of the same year the average of 42 examinations gave 71 per cubic centimetre. In Lake Geneva, Fol and Dunant obtained from water collected some distance from the shore an average of 38 bacteria per cubic centimetre.

Ice which is usually collected from lakes and rivers contains a greater or less number of bacteria, according to the depth and purity of the water. The ice used in Berlin, collected from the surface of lakes and rivers in the vicinity of the city, contains from a few hundred to 25,000 bacteria to the cubic centimetre (Fränkel). In the experiments of Heyroth samples of ice from the same source gave less than 100 per cubic centimetre in three, from 100 to 500 in eight, from 500 to 1,000 in six, from 1,000 to 5,000 in seven, and 14,400 in one.

Prudden obtained from Hudson River ice, put up six miles below the city of Albany, an average of 398 bacteria per cubic centimetre from transparent ice, and in the superficial "snow ice" 9,187. Ice collected lower down the river contained an average of 189 in the transparent and 3,693 in the snow ice.

Ice from the Dora at Turin was found by Bordoni-Uffreduzzi to contain from 120 to 3,546 bacteria per cubic centimetre.

Hydrant water, as supplied to cities, has received the attention of numerous investigators. The water supply of Berlin was examined by Plagge and Proskauer at intervals of a week from June, 1885, to April, 1886. Their tabulated results show considerable variations. We give the figures for a single day, June 30th, 1885: Stralauer works, water of the Spree, unfiltered 4,400, filtered 53; Tegeler works, water of the lake, unfiltered 880, filtered 44; high reservoir at Charlottenberg, 71; 75 W. Wilhelmstrasse, 121; Friedrichstrasse, 41–42 S. W., 160; Schmidstrasse, 165 E., 51; Friedrichstrasse, 126 N., 151; Weinmeisterstrasse, 15 C., 63.

Wells which are supplied by water from deep strata contain few bacteria, unless contaminated by surface water in which they are usually very abundant. Roth examined the water of sixteen surface wells in Belgard, which has a very porous subsoil, and found from 4,500 to 5,000 bacteria in three, from 7,800 to 15,000 in six, from 18,000 to 35,000 in six, and 130,000 per cubic centimetre in one.

Forty-seven wells in Stettin, the water of which was examined by Link, gave the following results: Less than 100 in six, 100 to 500 in twenty-one, and in the remainder (sixteen) from 1,000 to 18,000.

Sixty-four wells in Mainz examined by Egger, and 53 in Gotha by Becker, gave more favorable results; the number of wells in the former city, in which less than 100 colonies developed from 1 cubic centimetre, was 34, and in the latter the same (34). Bolton examined the water of 13 wells in Göttingen, and found but 1 in which the number of colonies from 1 cubic centimetre was less than 100; in 12 the number varied from 180 to 4,940.

The water of *deep wells* and *springs* may be entirely free from bacteria, or nearly so. Egger found in the water of an artesian well at Mainz 4 bacteria per cubic centimetre, and the same number was found by Hueppe in the deep well at the Wiesbaden slaughter-house. The artesian well at the gasworks of Kiel was found by Brennig to contain from 6 to 30 bacteria per cubic centimetre. In a spring at Batiolettes, Fol and Dunant found 57 bacteria per cubic centimetre. Fürbringer obtained from springs at Jena 156 from one, 51 from another, 32 from another, and 109 from another. The water supplied to Danzig from the Prangenaur Spring was found in several experiments to be free from bacteria (Freimuth).

In a summary of results obtained in various German cities Tiemann and Gärtner find that sixty-nine per cent of the wells from which samples of water were examined contained less than 500 bacteria per cubic centimetre.

The *water of sewers* is naturally rich in bacteria. Miquel found that at Clichy the sewer water contained 6,000,000 bacteria per cubic centimetre. Bischoff found in water from London sewers 7,500,000, and numerous observations show that the number of bacteria in river water is greatly increased in the vicinity of and below the mouths of city sewers.

We conclude from the experimental data recorded that water containing less than 100 bacteria to the cubic centimetre is presumably from a deep source and uncontaminated by surface drainage, and that it will usually be safe to recommend such water for drinking purposes, unless it contains injurious mineral substances. Water that contains more than 500 bacteria to the cubic centimetre, although it may in many cases be harmless, is to be looked upon with some suspicion, and water containing 1,000 or more bacteria is presumably contaminated by sewage or surface drainage and should be rejected or filtered before it is used for drinking purposes. But, as heretofore stated, the danger does not depend directly upon the number of bacteria present, but upon contamination with pathogenic species which are liable to be present in surface water and sewage. In swallowing a glassful of pure spring water a number of bacteria from the buccal cavity are washed away and carried into the stomach, which, if enumerated, would doubtless far exceed in numbers those found in the most impure river water.

The number of bacteria does not depend alone upon the amount of organic pabulum contained in a water, and cannot be depended upon in forming an estimate of this; for, as has been shown by Bolton, certain water bacteria multiply abundantly in water containing comparatively little organic matter, while other species fail to grow unless the quantity is greater. In a water containing considerable nutrient material the water bacteria may be restrained in their development by other species present until the amount of pabulum is reduced so that these no longer thrive, when the common water bacteria will take the precedence, and an enumeration may show a greater number of colonies than at first. But, in general, water rich in organic material contains a greater number of bacteria and a greater variety of species than that which is comparatively pure.

That certain bacteria may multiply in water which has been carefully distilled has been shown by Bolton and others. Two common water bacteria—Micrococcus aquatilis and Bacillus erythrosporus—multiplied abundantly in doubly distilled water, and when this water was again sterilized and re-inoculated with one of these species the same abundant increase occurred. This was repeated six times with the same result (Bolton). Computing the number of these water bacteria in ten cubic centimetres of distilled water at twenty millions, and estimating their specific gravity at one, and the diameter of the individual cells at one μ, the total weight of the entire number, according to Bolton, would be less than one-hundredth of a milligramme, and at least three-fourths of this must consist of water. The organic material represented by this number of bacteria would therefore be so minute that it might be supplied by dust particles accidentally falling into the distilled water.

Rosenberg has shown that while many of the species which he obtained in pure cultures from the water of the river Main multiplied in sterilized distilled water, other species quickly died out in such water. The growth of certain bacteria depends not only upon the quantity of nutritive material present, but upon its quality, the conditions in this regard being widely different for different species.

In view of the facts heretofore stated bacteriologists are now giving more attention to a careful study of the *kinds of bacteria present* in their examinations of water. Rosenberg, in his examinations of the water of the Main in the vicinity of Würzburg (1886), found that before the river reached the city the water contained more micrococci than bacilli, but that after receiving the sewage of the city the number of bacilli was greatly in excess.

Adametz (1888) has described eighty-seven species obtained by him from water in the vicinity of Vienna ; Maschek found fifty-five different species in the drinking water used at Leitmeritz; and Tils (1890) has described fifty-nine species obtained by him from the city water supply at Freiburg.

Among the *pathogenic bacteria* which are liable to find their way into water used for drinking purposes, the most important, from a sanitary point of view, are the bacillus of typhoid fever and the spirillum of Asiatic cholera. Both of these microörganisms are present in great numbers in the excreta of persons suffering from the specific forms of disease to which they give rise, and are consequently liable to contaminate wells and streams which receive surface water, when such excreta are thrown upon the surface or into sewers, etc. Epidemics of these diseases have frequently been traced to the use of such contaminated water, and in a few instances the presence of these specific disease germs in water has been demonstrated by bacteriological methods. Laboratory experiments indicate, however, that an increase of these pathogenic bacteria in drinking water is not likely to occur, except under special conditions, and that they die out after a time, being at a disadvantage in the struggle for existence constantly going on among the numerous species which have their normal habitat in water.

Bolton, Frankland, and others have shown that the anthrax bacillus, not containing spores, dies out in hydrant water within five or six days. In the experiments of Kraus the anthrax bacillus added to well water, not sterilized, at a temperature of 10.5° C., was still present in a living condition on the second day, but no colonies developed after the third day; the typhoid bacillus died out between the fifth and seventh days; the cholera spirillum was no longer found on the second day. In the meantime the common water bacteria had increased in numbers enormously. Similar results have been reported by Hochstetter and others. Hueppe, in ten experiments in which the typhoid bacillus was added to well water of a bad quality, found that in two no development of this bacillus occurred after the fifth day, while a few colonies developed in the other experiments as late as the tenth day. In these experiments the temperature was comparatively low (10.5° C.). At a higher temperature the experiments of Wolffhügel and Riedel show that an increase may take place. At the room temperature (about 20° C.) the typhoid bacillus added to distilled water, to well water, and to Berlin hydrant water was still present, in some instances, at the end of thirty-two days. And it was found that in some cases a decrease in the number occurred, then a notable increase, and finally a second diminution.

Koch found the cholera spirillum in a water tank at Calcutta during a period of fourteen days, and in his experiments showed that it preserved its vitality in well water for thirty days, in Berlin sewer water for six to seven days, and in the same mixed with fæces for twenty-seven hours only. In the experiments of Nicati and Rietsch the cholera spirillum preserved its vitality in distilled water for twenty days, in sewer water (of Marseilles) thirty-eight days, in water of the harbor for eighty-one days. The numerous experiments recorded by the observers named, and by Bolton, Hueppe, Hochstetter, Maschek, Kraus, and others, show that while the cholera spirillum may sometimes quickly die out in distilled water, in other experiments it preserves its vitality for several weeks (Maschek), and that it lives still longer in water of bad quality, such as is found in sewers, harbors, etc. Bolton found that for its multiplication a water should contain at least 40 parts in 100,000 of organic material, while the typhoid bacillus grew when the proportion was considerably less than this—6.7 parts in 100,000.

Russell (1891) has studied the bacterial flora of the Gulf of Naples, and of the mud at the bottom of this gulf, collected at various depths up to eleven hundred metres. His investigations show that sea water does not contain as many bacteria as an equal volume of fresh water; that bacteria are found in about equal numbers in water from the surface and in that from various depths; that the mud at the bottom constantly contains large numbers of bacteria; that some of the species isolated grow best in a culture medium containing sea water.

At a depth of 50 metres the water contained 121 bacteria per cubic centimetre, and the mud from the bottom 245,000 ; at 100 metres the water contained 10 and the mud 200,000 per cubic centimetre ; at 500 metres the water contained 22 and the mud 12,500 per cubic centimetre ; at 1,100 metres the mud contained 24,000.

The following new species were obtained by Russell from the source mentioned : Bacillus thalassophilus, Cladothrix intricata, Bacillus granulosus, Bacillus limosus, Spirillum marinum, Bacillus litoralis, Bacillus halophilus.

The bacterial flora of fresh and sea water is very extensive, as will be seen by the following list of species which have been described by various bacteriologists who have given their attention to its study :

NON-PATHOGENIC MICROCOCCI.

Micrococcus aurantiacus (Cohn), Micrococcus luteus (Cohn), Micrococcus violaceus (Cohn), Micrococcus flavus liquefaciens (Flügge), Micrococcus flavus desidens (Flügge), Micrococcus radiatus (Flügge), Micrococcus cinnabareus (Flügge), Micrococcus flavus tardigradus (Flügge), Micrococcus versicolor (Flügge), Micrococcus agilis (Ali-Cohen), Micrococcus fuscus (Maschek), Diplococcus luteus (Adametz), Pediococcus albus (Lindner), Micrococcus cerasinus siccus (List), Micrococcus citreus (List), Micrococcus aquatilis (Bolton), Micrococcus fervidosus (Adametz), Micrococcus plumosus (Bräutigam), Micrococcus viticulosus (Katz), Micrococcus cremoides (Zimmermann), Micrococcus carneus (Zimmermann), Micrococcus concentricus (Zimmermann), Micrococcus rosettaceus (Zimmermann), Micrococcus ureæ (Pasteur), Weisser Streptococcus (Maschek), Wurmförmiger Streptococcus (Maschek), Micrococcus aërogenes (Miller), Sarcina alba, Sarcina candida (Reinke), Sarcina lutea.

PATHOGENIC MICROCOCCI.

Staphylococcus pyogenes aureus (Rosenbach), Micrococcus of Heydenreich—"Micrococcus Biskra."

NON-PATHOGENIC BACILLI.

Bacillus arborescens (Frankland), Bacillus viscosus (Frankland), Bacillus aquatilis (Frankland), Bacillus liquidus (Frankland), Bacillus nubilis (Frankland), Bacillus vermicularis (Frankland), Bacillus aurantiacus (Frankland), Bacillus cœruleus (Smith), Bacillus glaucus (Maschek), Bacillus albus putidus (Maschek), Bacillus fluorescens liquefaciens, Bacillus fluorescens nivalis (Schmolck), Bacillus lividus (Plagge and Proskauer), Bacillus rubidus (Eisenberg), Bacillus sulfureum (Holschewnikoff), Bacillus violaceus, Bacillus gasoformans (Eisenberg), Bacillus liquefaciens (Eisenberg), Bacillus phosphorescens indicus (Fischer), Bacillus phosphorescens indigenus (Fischer), Bacillus phosphorescens gelidus (Katz), Bacillus smaragdino-phosphorescens (Katz), Bacillus argenteo-phosphorescens Nos. I., II., and III. (Katz), Bacillus cyaneo-phosphorescens (Katz), Bacillus argenteo-phosphorescens liquefaciens (Katz), Bacillus ramosus, Bacillus subtilis (Ehrenberg), Proteus sulfureus (Lindenborn), Bacillus aureus (Adametz), Bacillus brunneus (Adametz), Bacillus flavocoriaceus (Adametz), Bacillus fluorescens non-liquefaciens, Bacillus latericeus (Adametz), Bacillus stolonatus (Adametz), Bacillus berolinensis indicus (Clässen), Bacillus erythrosporus (Eidam), Bacillus luteus (List), Bacillus aquatilis sulcatus Nos. 1, 2, 3, 4, and 5 (Weichselbaum), Bacillus albus (Eisenberg), Bacillus multipediculosus (Flügge), Bacillus Zürnianum (List), Bacillus fulvus (Zimmermann), Bacillus helvolus (Zimmermann), Bacillus ochraceus (Zimmermann), Bacillus plicatus, Bacillus devorans (Zimmermann), Bacillus gracilis (Zimmermann), Bacillus guttatus (Zimmermann), Bacillus implexus (Zimmermann), Bacillus punctatus (Zimmermann), Bacillus radiatus aquatilis (Zimmermann), Bacillus vermiculosus (Zimmermann), Bacillus constrictus (Zimmermann), Bacillus fluorescens aureus (Zimmermann), Bacillus fluorescens longus (Zimmermann), Bacillus fluorescens tenuis (Zimmermann), Bacillus fuscus (Zimmermann), Bacillus rubefaciens (Zimmermann), Bacillus subflavus (Zimmermann), Bacillus janthinus (Zopf), Bacillus mycoides (Flügge), Bacillus tremelloides (Tils), Bacillus cuticularis (Tils), Bacillus filiformis (Tils), Bacillus ubiquitus (Jordan), Bacillus circulans (Jordan), Bacillus superficialis (Jordan), Bacillus reticularis (Jordan), Bacillus rubescens (Jordan), Bacillus hyalinus (Jordan), Bacillus cloacæ (Jordan), Bacillus delicatulus (Jordan), Bacillus violaceus laurentius (Jordan).

PATHOGENIC BACILLI.

Bacillus typhi abdominalis (Eberth, Gaffky), Bacillus erysipelatos suis ("Bacillus murisepticus," Koch), Bacillus septicæmiæ hæmorrhagicæ ("Bacillus cuniculicida," Koch), Proteus vulgaris (Hauser), Proteus mirabilis (Hauser), Bacillus canalis capsulatus (Mori), Bacillus canalis parvus (Mori), Spirillum choleræ Asiaticæ ("Comma bacillus," Koch), Bacillus coli communis (Escherich), Bacillus hydrophilus fuscus (Sanarelli), Bacillus venenosus (Vaughan), Bacillus venenosus brevis (Vaughan), Bacillus venenosus invisibilis (Vaughan), Bacillus venenosus liquefaciens (Vaughan).

The following additional species are described by Zimmermann (1894) in his second publication ("Die Bakterien unserer Trink- und Nutzwässer"). Micrococcus candidus, Micrococcus coralloides, Streptococcus cinereus, Micrococcus sulphureus, Micrococcus galbanatus, Micrococcus erythromyxa, Sarcina flavea, Sarcina aurantiaca, Sarcina rosea. Bacillus ruber, Bacillus miniaceus, Bacillus mesentericus roseus, Bacillus carnosus, Bacillus chrysogloia, Bacillus multipediculus flavus, Bacillus villosus, Bacillus radiatus, Bacillus fluorescens albus, Bacillus viridans, Bacillus turcosa, Bacillus halans, Bacillus nacreaceus, Bacillus mirabilis, Bacillus umbilicatus, Bacillus lactis viscosus, Bacillus synxanthus, Bacillus sericeus, Bacillus minutus, Bacillus stellatus, Bacillus radicosus, Bacillus vernicosus, Bacillus mucosus, Bacillus centralis, Bacillus spumosus, Bacillus annulatus, Bacillus liquefaciens, Bacillus disciformans.

The following spirilla and "vibrios" have also been found in water—chiefly in river water:

Spirillum volutans, Spirillum sanguineum, Spirillum serpens, Vibrio rugula, Spirillum plicatile, Spirillum marinum (Russell). Spirillum choleræ Asiaticæ, Spirillum of Rénon, Vibrio aquatilis (Gunther), Vibrio of Weibel, Vibrios of Bujwid (Bacillus choleroides *a* and *b*), Vibrio of Löffler, Vibrios of Bonhoff, Vibrio of Blackstein, Vibrios of Sanarelli, Vibrios of Fischer, Vibrio Berolinensis, Vibrio Danubicus, Vibrio of Pfuhl (v. Metchnikovi ?). Several of the "vibrios" in this list which have recently been obtained from river water in various parts of Europe are probably varieties of the cholera spirillum.

ADDITIONAL NOTES UPON BACTERIA IN WATER.

It is now generally recognized by bacteriologists that the potability of water is to be determined by an investigation relating to the presence or absence of known pathogenic bacteria, rather than by an estimate of the number of bacteria present in each cubic centimetre of the water under examination. From a sanitary point of view the most important of these pathogenic bacteria are the cholera spirillum and allied "vibrios," the bacilli of the "typhoid group" (Bacillus typhi abdominalis and allied forms), the bacilli of the "colon group" (Bacillus coli communis with its varieties and similar bacilli of fæcal origin). When one of these pathogenic bacilli is present in a water-supply in small numbers as compared with the number of saprophytic bacteria, it is not an easy matter to demonstrate the fact by the ordinary plate method, especially in the case of non-liquefying species like the typhoid bacillus. If we have, for example, one typhoid bacillus to one thousand bacilli of other species it is evident that in a series of three plates, made in the usual way for the purpose of obtaining isolated colonies, there would be but a small chance of obtaining a colony of the typhoid bacillus in plate No. 3, and a plate containing one thousand colonies or more would be so crowded that the detection of the single typhoid colony would be very difficult. For this reason, it is necessary to resort to special methods by which the more numerous saprophytic bacteria will be excluded, or their numbers greatly reduced. Some of the methods which have been successfully employed for the detection of the typhoid bacillus and of the cholera spirillum are given in the sections devoted to these microörganisms. We give below some details relating to the methods employed by bacteriologists of recognized competence in recent investigations :

Marpmann (1895) considers all water which contains fæcal bacteria as dangerous as a supply for drinking purposes. For the detection of pathogenic bacteria he recommends the following procedure :

The pathogenic bacteria are divided into two groups by cultivation in nutrient agar containing 0.2 per cent of citric acid, and in the same medium containing two per cent of sodium carbonate. The bacilli of the typhoid group are said to grow in the acid medium but not in that containing two per cent of sodium carbonate. On the other hand, cholera vibrios develop in the alkaline medium but not in that containing 0.2 per cent of citric acid. The bacilli of the colon group also ("cloaca-bacilli") do not grow in the medium containing citric acid. Bouillon containing the same amounts of acid and alkali is also employed. The water to be examined is first mixed with an equal portion of acid and of alkaline bouillon in two test tubes, and these are kept at a temperature of 30° C. for twenty-four hours, during which time the pathogenic bacteria, if present, will multiply and cause a clouding of the culture media. Inoculations are now made into the acid and alkaline agar and gelatin. Growth in alkaline gelatin at the room temperature (10° to 18° C.) is due to "cloaca-bacteria" ; growth in acid gelatin at 20° to 23° C. is due to bacilli of the typhoid group. Plates should also be made from the clouded bouillon, acid and alkaline ; and the colonies resembling those of the typhoid or of the colon group should be tested in nutrient gelatin containing sugar to ascertain whether there is development of gas, in which case the bacilli are of the colon group.

When typhoid and colon bacilli are associated in water the last-mentioned bacillus takes the precedence, and the typhoid bacillus has a tendency to disappear. This is shown by the experiments of Gimbert (1894), who introduced, at the same time, colon bacilli and typhoid bacilli into water, and found that at the end of forty-eight hours he was no longer able to isolate the typhoid bacillus from plates. In view of this fact failure to find the typhoid bacillus does not relieve the water from the suspicion of being dangerous if the colon bacillus is present. But, on the other hand, this bacillus is so common that it is perhaps the exception when it is not present in surface waters. As pointed out by von Freudenreich (1895) it may, however, escape detection unless a considerable quantity of water is used in making the test. When the quantity is from one hundred to five hundred cubic centimetres, instead of from one to five cubic centimetres, as was formerly the usual amount employed, it is found not infrequently even in spring water (von Freudenreich).

The author last mentioned says that when present in small numbers it may be demonstrated by the method of Vincent, as follows : Mix of the water ninety cubic centimetres with ten cubic centimetres of a twenty-per-cent solution of peptone, and one cubic centimetre of a seven-per-cent solution of carbolic acid ; place in the incubating oven at 42° C. If development occurs it will probably be due to the colon bacillus, but it will be necessary to make plates and pure cultures from single colonies in order to determine this with certainty. The demonstration may be made more quickly, according to von Freudenreich, by using a medium containing milk sugar (five per cent) and cultivating at 35° C. If the colon bacillus is present there will be an abundant development of gas in from twelve to twenty-four hours, and the bacillus may then be readily isolated by the plate method. The colon bacillus has been found by Moissan and Gimbert in mineral waters bottled in France. Poncet (1895) has made a careful study of the bacteria found in the various springs at Vichy. The species described are all harmless water bacteria and have little interest from a sanitary point of view.

Kruse (1894), as a result of his extended researches and of a critical consideration of the experimental data available, arrives at the conclusion that a sanitary inspection of the sources of supply is more important, in determining the safety of the supply from a sanitary point of view, than a chemical or bacteriological examination. The writer has for some years past entertained the same opinion. Kruse says, however, that for the control of filtering plants bacteriological "counting-methods" are indispensable. He also ascribes a "high scientific value" to investigations relating to the presence of the more important pathogenic bacteria; but says that, notwithstanding the improvements in methods of research, we cannot wait for a demonstration of the presence of the cholera or typhoid bacteria before condemning a water as probably unsafe, if sources of contamination are discovered—or, we would add, if cases of cholera or typhoid fever can be traced with a fair degree of certainty to the use of water from a given source.

Fischer (1894), in his account of the researches made during the Plankton expedition, has given a summary of the experimental evidence relating to the presence of bacteria in the waters of the ocean. The species found were for the most part different from those found in lakes and rivers, and at some distance from the shore none of the previously known species of micrococci and bacilli were encountered. The number of bacteria in samples from the surface at a distance from the shore was comparatively small (usually less than five hundred per cubic centimetre), but in the vicinity of land very large numbers were sometimes found. At a distance of ten metres below the surface the number found was greatly in excess of the number at the surface —the difference being probably due to the germicidal action of sunlight. At depths of four hundred metres bacteria were constantly found in great numbers, and water from a depth of eleven hundred metres was still found to contain them.

III.

BACTERIA IN THE SOIL.

SURFACE soil, and especially that which is rich in organic matter, contains very numerous bacteria of many different species. Some of these are of special interest on account of their pathogenic power. Thus the bacillus of malignant œdema and the bacillus of tetanus have been shown to be widely distributed species, which have been obtained by investigators in various parts of the world by inoculating susceptible animals—guinea-pigs or mice—with a little rich surface soil. Other species are interesting because of their action in nitrification and in the destructive decomposition of organic material by which it is fitted for assimilation by the higher plants. Many of the bacteria present in the soil are strictly anaërobic, and in attempts to estimate the number and kind of microörganisms present in a given sample this fact must be kept in view.

The simplest *method of studying* the bacteria in the soil consists in introducing a small quantity into liquefied gelatin in test tubes, and, after carefully crushing it with a sterilized glass rod and thoroughly mixing it with the gelatin, making roll tubes in the usual way. Some of these should be put up for anaërobic cultures—*i.e.*, the tube should be filled with an atmosphere of hydrogen according to Fränkel's method. If the object in view is to estimate the number of bacteria in a given sample of soil the difficulty is encountered that, however finely crushed, the little masses of earth are likely to contain numerous bacteria, and we cannot safely assume that each colony originates from a single germ. Thoroughly washing a small quantity of soil, by agitation, in a considerable quantity of distilled water, and then adding a definite quantity of the water to nutrient gelatin and making roll tubes or plates, as in water analysis, suggests itself as a simple method ; but Fränkel has shown that it is far from being reliable when the object is to estimate the number of bacteria. He obtained more uniform and accurate results by introducing the earth at once into liquefied gelatin and crushing it as thoroughly as possible with a strong platinum wire, after which as thorough a mixture as possible was effected by tilting the tube up and down. But for the purpose of obtaining pure cultures from single colonies of the various species present, we should prefer to wash the earth in distilled water and to allow the sediment to settle before taking a portion of the water to add to the nutrient medium.

In some experiments made in 1881 Koch ascertained that in soil which had not been disturbed but few bacteria were to be found at the depth of a metre; and this fact has since been established by the extended researches of Fränkel, who devised a special boring instrument for obtaining samples of earth from different depths. Miquel, in 1879, estimated the number of bacteria in one gramme of earth collected in the park of Montsouri, Paris, at a depth of twenty centimetres, at 700,000; and in a cultivated field which had been treated with manure, at 900,000. The following results were obtained by Adametz : One gramme of earth from a sandy soil contained at the surface 380,000, at a depth of twenty to twenty-five centimetres 400,000; the same quantity of clayey soil contained at the surface 500,000, at a depth of twenty to twenty-five centimetres 460,000.

In experiments made by Beumer (1886) and by Maggiora (1887) considerably greater numbers were found, but the last-named observer, in some instances at least, kept the earth for some time after collecting it, which may have materially influenced the result. Beumer obtained from a specimen of sandy humus taken from a depth of three metres 45,000,000 to the gramme; at four metres, 10,000,000; at five metres, 8,000,000; at six metres, 5,000,000. These specimens were obtained from the vicinity of hospitals at Greifswald. In a churchyard, at a depth of four metres, the number in one experiment was 1,152,000, and in another 1,278,000.

Fränkel has given special attention to the examination of undisturbed soil not in the immediate vicinity of dwellings. In samples from a fruit orchard near Potsdam he found that the superficial layers contained from 50,000 to 350,000 germs per cubic centimetre. The greatest number was not immediately upon the surface, but at from one-quarter to one-half metre below the surface. The number was found to be greater in summer than in winter, the maximum being in July and August. At a depth of three-quarters of a metre to a metre and a half there was a very great and abrupt diminution in the number of germs. From 200,000 at one-half metre the number fell to 2,000 at a depth of a metre, from 250,000 at three-quarters of a metre to 200 at one metre, etc., and at a depth of one and one-half metres, in some instances, no more living germs were obtained. In other experiments a few colonies developed from earth obtained at a depth of three or four metres, but these were slow in making their appearance, and often several days, or even weeks, elapsed before they became visible in Esmarch roll tubes. In experiments with surface soil, on the contrary, a multitude of colonies developed within twenty-four to forty-eight hours, and, as many liquefying bacteria were present, it was necessary to make the enumeration on the first or second day, at which time, no doubt, many of the bacteria present had not yet formed visible colonies. The results obtained have, therefore, only a relative value.

The most important fact developed by Fränkel's researches is that in virgin soil there is a dividing line at a depth of from three-quarters to one and one-half metres, below which very few bacteria are found, and that, consequently, the "ground-water region" is free from microörganisms, or nearly so, notwithstanding the immense numbers present in the superficial layers.

The extended researches of Maggiora, made in the vicinity of Turin, led him to the following conclusions:

> 1. The number of germs in desert and forest soils is much smaller, other conditions being equal, than in cultivated lands, and in these it is less than in inhabited localities.
> 2. In desert soils the number of germs bears a relation (*a*) to the geological epoch to which the lands belong, and, within certain limits, to the height above the level of the sea—the older the soil and the greater the altitude, other things being equal, the fewer the germs; (*b*) to the compactness and aëration of the soil—the more compact and impermeable to air the smaller the number of germs capable of developing in gelatin; (*c*) to the nature of the soil—sandy soils contain fewer germs than soils rich in clay and in humus.
> 3. In cultivated lands the number of germs augments with the activity of cultivation and the strength of the fertilizers used.
> 4. In inhabited localities the number of germs in the superficial layers is very great. In the deep layers it usually diminishes rapidly, as is the case in all other soils.

As to the kinds of bacteria present, and their biological characters and functions in preparing organic material for assimilation by the plants whose roots penetrate the soil, we have yet much to learn. Fränkel remarks that the species most frequently encountered in the deeper strata of the soil were three bacilli which also abound in the superficial layers—viz., the "hay bacillus," the "wurzel bacillus," and the "hirnbacillus." In all eleven bacilli were isolated and cultivated. Micrococci were only found four times, and spirilla not at all. Mould fungi were more abundant, and especially one previously obtained from the air by Hesse and called by him "brauner Schimmelpilz." Anaërobic bacilli, contrary to expectation, were not obtained in Fränkel's researches, and no pathogenic species were found in the deeper layers of the soil. We have already referred to the fact that the bacillus of malignant œdema and the bacillus of tetanus, two pathogenic, anaërobic species, are common in rich surface soil in various parts of the world.

The results obtained in the researches referred to, in which nutrient gelatin was used as a culture medium, are no doubt very incomplete, not only on account of the liquefaction of the gelatin by common liquefying bacilli before other species present have formed visible colonies, but also because this is not a favorable culture medium for some of the species present in the soil. Thus Frankland has succeeded in isolating a nitrifying ferment which he calls "Bacillococcus," which grows abundantly in bouillon, but fails to grow in nutrient gelatin. Winogradski has also obtained in pure cultures a nitrifying ferment from the soil in the vicinity of Zurich, which he has called "Nitromonas."

Comparatively few micrococci are found in the soil, while in the air they are usually found to be more abundant than bacilli. This is perhaps due to the fact that the bacilli are more promptly destroyed by desiccation and the action of sunlight.

Several bacteriologists have made investigations relating to the duration of vitality of pathogenic bacteria in the soil. Fränkel found that in Berlin the bacillus of anthrax, in Esmarch roll tubes, when buried in the soil at a depth of two metres, only occasionally gave evidence of growth, and at three metres no development occurred. The comparatively low temperature at this depth was no doubt an important factor in influencing the result. The cholera spirillum in the months of August, September, and October grew at a depth of three metres, but in the remaining months of the year failed to grow at two, while growth occurred at one and one-half metres. The bacillus of typhoid fever grew at three metres during the greater portion of the year.

Giaxa has made extended and interesting experiments with the cholera spirillum, cultures of which he added to different kinds of soil (garden earth, clay, sand) and placed at different depths below the surface—one-quarter, one-half, and one metre. Some of the earth was sterilized and some was not. In the unsterilized earth he found the cholera spirillum in considerable numbers at the end of twenty-four hours at the greatest depth tested (one metre), but at the end of forty eight hours it had disappeared in five experiments out of seven—the lowest temperature at this depth was 20° C. In the sterilized soil the result was different; the cholera spirillum was present in enormous numbers at the end of four days at a depth of a metre, and was still found in smaller numbers at the end of twelve days, but had disappeared at the end of twenty-one days. These results indicate that the presence of common saprophytes in the soil is prejudicial to the development of the cholera spirillum, and that under ordinary circumstances it succumbs in the struggle for existence with these more hardy microörganisms.

The researches of Proskauer (1891) confirm those of Fränkel and others as to the rapid diminution in the number of bacteria in the deeper layers of the soil. They also agree with those of Gärtner in showing that in the soil of churchyards the number of bacteria diminishes greatly in the soil beneath the layer containing coffins. In general the influence of dead bodies upon the bacteria in the soil in the vicinity of coffins was very slight; in the subsoil of the graveyard there were not many more bacteria than in similar soil outside of this. Reimers had previously shown that samples of earth from two graves, in one of which the body had been buried for thirty-five years and in the other for one and one-half years, gave similar results when examined by bacteriological methods.

Manfredi in 1892 published the results of his extended investigations relating to the dust in the streets of Naples. The number of bacteria varied greatly in different parts of the city. In streets where the traffic was least and hygienic conditions the best the average number was 10,000,000 per gramme. In dirty and busy thoroughfares the average was 1,000,000,000, and in certain localities the number was even five times as great as this. Injections into guinea-pigs gave a positive result in seventy-three per cent of the animals experimented upon. Among the known pathogenic bacteria obtained in this way were the pus cocci (in eight), Bacillus tuberculosis (in three), the bacillus of malignant œdema, and the tetanus bacillus.

In the memoir of Fülles (1891) the following species are described as having been found by him in the soil at Freiburg, Germany:

MICROCOCCI.

(*a*) *Non-liquefying.*—Micrococcus aurantiacus (Cohn), Micrococcus candidus (Cohn), Micrococcus luteus (Cohn), Micrococcus candicans (Flügge), Micrococcus versicolor (Flügge), Micrococcus cinnabareus (Flügge), Micrococcus cereus albus (Passet), Micrococcus fervitosus (Adametz), Rother coccus (Maschek).

(*b*) *Liquefying.*—Micrococcus flavus liquefaciens (Flügge), Micrococcus flavus desidens (Flügge), Diplococcus luteus (Adametz), Sarcina lutea.

NON-PATHOGENIC BACILLI.

(*a*) *Non-liquefying.*—Bacillus fluorescens putidus (Flügge), Bacillus muscoides (Liborius), Bacillus scissus (Frankland), Bacillus candicans, Bacillus diffusus (Frankland), Bacillus filiformis (Tils), Bacillus luteus (Flügge), Fluorescent water bacillus (Eisenberg), Bacillus viridis pallescens (Frick), Bluish-green fluorescent bacillus (Adametz), Bacillus stolonatus (Adametz), Bacillus Zürnianum (List), Bacillus aërogenes (Miller), Bacillus No. 1 and Bacillus No. 2 (Fülles).

(*b*) *Liquefying.*—Bacillus ramosus liquefaciens (Flügge), Bacillus liquidus (Frankland), Bacillus ramosus—"wurzel bacillus," Bacillus subtilis

(Ehrenberg), Bacillus mesentericus fuscus (Flügge), Bacillus mesentericus vulgatus (Flügge), Bacillus fluorescens liquefaciens (Flügge), Lemon-yellow bacillus (Maschek), Green yellow bacillus (Eisenberg), Gas-forming bacillus (Eisenberg), Gray bacillus (Maschek), Bacillus prodigiosus (Ehrenberg), Proteus mirabilis (Hauser), Proteus vulgaris (Hauser), Bacillus mesentericus vulgatus, Bacillus cuticularis (Tils), " Weisser bacillus " (Eisenberg).

(*c*) *Pathogenic.*—Bacillus œdematis maligni (Koch).

In addition to the above the following species have been described by other authors: Bacillus liquefaciens magnus (Lüderitz), Bacillus radiatus (Lüderitz), Bacillus solidus (Lüderitz), Bacillus mycoides roseus (Scholl), Bacillus viscosus (Frankland), Bacillus candicans (Frankland), Bacillus poliformis (Liborius), Clostridium fœtidum (Liborius).

Pathogenic species.—Staphylococcus pyogenes aureus (Rosenbach), Bacillus tetani (Nicolaier), Streptococcus septicus (Nicolaier), Pseudo-œdema bacillus (Liborius), Bacillus septicus agrigenus (Nicolaier), Bacillus of Utpadel.

IV.

BACTERIA OF THE SURFACE OF THE BODY AND OF EXPOSED MUCOUS MEMBRANES.

Great numbers of bacteria of various species multiply upon the *surface of the human body,* where they find the necessary pabulum in the excretions from the skin and the exfoliated epithelium. Evidently the number will be largely influenced by the clothing worn, the atmospheric conditions as to heat and moisture, personal habits, etc. The writer has frequently inoculated culture media with a drop of sterilized fluid which had been placed upon the surface of the body of patients in hospitals and of healthy persons. By friction with a platinum needle at the point where the drop of fluid is applied the surface is washed and a little epithelium detached. Cultures may always be obtained by inoculating nutrient media from a drop of fluid applied in this way. Micrococci of various species, including the pus cocci, are very commonly encountered ; sarcinæ and various bacilli are also frequently met with. Even the hands, which by reason of their exposure and frequent ablutions are freer from exfoliated epithelium than portions of the body covered with clothing, have constantly attached to their surface a considerable number of bacteria. This is shown by the experiments of Kümmel and Forster, of Fürbringer and others, with reference to the disinfection of the hands. Forster found that after the most careful cleaning of the hands with soap, water, and a brush, contact of the fingers with nutrient gelatin always resulted in the development of a greater or less number of colonies.

Bordoni-Uffreduzzi, in his researches relating to the bacteria of the skin, obtained in pure cultures five different species of micrococci and two bacilli. Pure cultures of his Bacterium graveolens, which was usually found between the toes, gave off a disagreeable odor like that observed from this locality in certain individuals. In his researches made in Havana the writer frequently encountered in cultures from the surface, associated with various micrococci, his Micrococcus tetragenus versatilis.

Fürbringer found quite frequently in the spaces beneath the finger nails Staphylococcus pyogenes aureus associated with various other microörganisms. A similar result had previously been reported by Bockhart.

In his examinations of water from various sources Miquel found that "wash-water" from the floating laundries on the Seine contained more bacteria than water from any other source, even than the water of the Paris sewers. His enumeration gave twenty-six million germs per cubic centimetre.

Hohein has enumerated the colonies developing from underclothing worn for various lengths of time and made of different kinds of material. A piece of the goods to be tested was sewed fast to the underclothing, so as to come in immediate contact with the body ; at the end of a given time a fragment one-quarter of a centimetre square was cut up as fine as possible and distributed in nutrient gelatin. Plates were made and the colonies counted at the end of five or six days.

In an experiment in which sterilized woven goods were worn next to the skin of the upper arm the following results were obtained : Linen goods, at the end of one day 28, two days 4,180 colonies ; cotton goods, end of one day 105, end of two days 1,870 ; woollen goods, end of one day 606, end of two days 6,799. When the material had been in contact with the skin for four days the colonies which developed were so numerous that they could not be counted.

Maggiora isolated twenty-two species of bacteria from his cultures inoculated with epidermis from the foot. None of these proved to be pathogenic for mice, rabbits, or guinea-pigs. Several gave off a strong odor of trimethylamin, similar to that of sweating feet.

The following species have been found upon the surface of the body :

Non-pathogenic.—Diplococcus albicans tardus (Unna and Tommasoli), Diplococcus citreus liquefaciens (Unna and Tommasoli), Diplococcus flavus liquefaciens tardus (Unna and Tommasoli), Staphylococcus viridis flavescens (Guttmann), Bacillus graveolens (Bordoni–Uffreduzzi), Bacillus epidermidis (Bordoni), Ascobacillus citreus (Unna and Tommasoli), Bacillus fluorescens liquefaciens minutissimus (Unna and Tommasoli), Bacillus aureus (Unna and Tommasoli), Bacillus ovatus minutissimus (Unna and Tommasoli), Bacillus albicans pateriformis (Unna and Tommasoli), Bacillus spiniferus (Unna and Tommasoli), Bacillus of Scheurlen, Micrococcus tetragenus versatilis (Sternberg), Bacillus Havaniensis liquefaciens (Sternberg).

Pathogenic.—Staphylococcus pyogenes albus, Staphylococcus pyogenes aureus, Streptococcus pyogenes, Diplococcus of Demme, Bacillus of Demme, Bacillus of Schimmelbusch, Bacillus of Tommasoli, Bacillus saprogenes II. (Rosenbach), Bacillus parvus ovatus (Löffler).

SURFACE OF MUCOUS MEMBRANES.

Cultures made from the *conjunctivæ* of healthy persons usually show the presence of various micrococci, and sometimes of bacilli.

McFarland (1895) says that in his researches the microörganisms found were for the most part "those already described by others and of common occurrence in the air." He encountered, however, several bacilli not previously described ("Bacillus hirsutus, Bacillus cœrulefaciens, Bacillus circumscriptus, Bacillus succinacius, Bacillus violaceus flavus"). Lachowicz (1895) failed to obtain any bacteria in his cultures from the conjunctival sac in sixty-nine per cent of the healthy eyes examined by him (sixty-three eyes in all). He concludes that the microörganisms, which at times are found in the healthy conjunctival sac, come principally from the air; that they are present in small numbers and probably remain only for a short time. His experiments show that most species when artificially introduced rapidly diminish in numbers and soon disappear entirely. Cultures of Streptococcus pyogenes and of Bacillus xerosis conjunctivæ introduced into healthy eyes did not cause the slightest irritation. In this connection we may remark that the same is true as regards pathogenic bacteria introduced into the bladder, but that when there is some cause of local irritation or injury a chronic cystitis is likely to be developed. In like manner, we believe, chronic conjunctivitis may be developed as the result of local irritation in connection with the presence of pathogenic bacteria and especially of the pyogenic micrococci.

The extended researches of Bach (1894) gave results corresponding with those of previous investigators, and not with those reported by Lachowicz, who, as stated above, failed to obtain cultures from sixty-nine per cent of the healthy eyes examined. Bach says: "In a large percentage of the cases the presence of bacteria may be demonstrated, even when the conjunctiva presents a perfectly normal appearance; the conjunctival sac must therefore be regarded as constantly infected." Bach describes twenty-seven different microörganisms obtained by him in pure cultures from this source, of these eighteen are micrococci. He recognizes the fact that most of them come from the air, while others are introduced by the hands in rubbing the eyes, etc. In diseased conditions these are more numerous than in health, but the pus cocci are not infrequently found in healthy eyes.

As bacteria are constantly present in the air, they are necessarily deposited upon the moist mucous membrane of the *nose* during inspiration. Indeed, it would appear as if an important function of this extended mucous membrane is to purify the air from suspended particles, and it has been shown by experiment that expired air is practically free from bacteria. The greater number of those contained in inspired air are deposited upon the mucous membrane of the anterior nares. In culture experiments made by Von Besser, Wright, and others the nasal mucus was found to contain a great variety of bacteria; among others the pus cocci were frequently found by both of the observers mentioned. In eighty one cases Von Besser found the "diplococcus pneumoniæ" fourteen times, Staphylococcus pyogenes aureus fourteen times, Streptococcus pyogenes seven times, and Friedländer's bacillus twice. Twenty-eight of the cases examined were convalescents in hospital; among these the pathogenic species mentioned were found less frequently than in other individuals. The following non-pathogenic species were isolated: Micrococcus liquefaciens albus in twenty-two cases, Micrococcus albus in nine cases, Micrococcus cumulatus tenuis in fourteen cases, Micrococcus flavus liquefaciens in three cases, Bacillus striatus albus in ten cases, etc.

Paulsen (1890) made thirty-one cultures in nutrient gelatin from sixteen persons and thirty-three in nutrient agar from twenty-two persons, with the following result: Eleven remained sterile, nineteen showed not more than ten colonies, sixteen less than one hundred, twelve more than one hundred, and in six the number was so great that they could not be counted. Micrococci were more numerous than bacilli; of these a "sulphur-yellow coccus" in tetrads was found in eight individuals. Various species of liquefying cocci, resembling the pus cocci, were isolated, but the conclusion was reached that none of these were identical with the staphylococci of pus, which Von Besser and Wright both found in a considerable proportion of the culture experiments made by them.

Thomson and Hewlett (1895) have recently reported results which differ to some extent from those previously reported. While they found numerous bacteria in the vestibulum naris, cultures made from mucus obtained from the interior of the nose usually gave a negative result—sixty-four out of seventy-six remained absolutely sterile, while in seven there was a scanty growth only. They conclude that while microörganisms are occasionally found upon the Schneiderian membrane they are not numerous and are often entirely absent; and that they are rarely found upon the pituitary membrane. Straus (1895) has examined the nasal secretions of persons associated with tubercular patients for the purpose of ascertaining if the tubercle bacillus was present. The presence of this bacillus was demonstrated, by inoculation into guinea-pigs, in nine healthy individuals out of twenty-nine examined; two of these were physicians and six were nurses.

Very extended researches have been made with reference to the bacteria present in the human *mouth*, which show that numerous species are constantly present in the buccal secretions and upon the surface of the moist mucous membrane. Some of these are occasional and accidental, while others appear to have their normal habitat in the mouth, where the conditions as to temperature, moisture, and presence of organic pabulum are extremely favorable for their development. A minute drop of saliva spread upon a glass slide, dried, and stained with one of the aniline colors, will always be found to contain an immense number of bacteria of various forms. Some of these are attached to epithelial cells and some scattered about singly or in groups. Among those seen in a single specimen we will usually find cocci in tetrads, in chains, and in irregular groups, bacilli of various dimensions, and occasionally spirilla. According to Prof. Miller, of Berlin, the following species almost invariably occur in every mouth: Leptothrix innominata, Bacillus buccalis maximus, Leptothrix buccalis maxima, Iodococcus vaginatus, Spirillum sputigenum, Spirochæte dentium. All of these fail to grow in ordinary culture media. Miller has made extended attempts to obtain cultures by varying the medium used and attempting to imitate as nearly as possible the natural medium in which they are found; but his attempts have been unsuccessful, or nearly so—"only line cultures afforded a limited growth, but the colonies never developed more than fifteen to twenty cells, and a transference to a second plate proved futile, no further growth taking place."

Up to the year 1885 Miller had isolated twenty-two different species of bacteria from the human mouth. Ten of these were cocci, five short bacilli, six long bacilli, and one a spirillum. Later the same author cultivated eight additional species. Vignal has isolated and described seventeen species obtained by him in pure cultures from the healthy human mouth; most of these are bacilli, and Miller, who found micrococci to be more numerous, supposes the difference in results to be due to the fact that many of the cocci do not grow in nutrient gelatin, which was the medium employed by Vignal. In the researches of the last-named author the following species were obtained most frequently, in the order given: 1. Bacterium termo. 2. Bacillus *e* (Bacillus ulna ?). 3. Potato bacillus. 4. Coccus *a*. 5. Bacillus *b*. 6. Bacillus *d*. 7. Bacillus *c* (Bacillus alvei ?). 8. Bacillus subtilis. 9. Staphylococcus pyogenes albus. 10. Staphylococcus pyogenes aureus.

Among the species above enumerated we find two of the most common pus cocci, Staphylococcus albus and aureus, but no mention is made of another important pathogenic micrococcus which is frequently found in the healthy human mouth, viz., the micrococcus of sputum septicæmia, first named by the writer Micrococcus Pasteuri. This does not grow at ordinary temperatures, and consequently would not be obtained in gelatin plate cultures. Very different results have been reported by different observers as to the frequency with which the pathogenic cocci are found in the buccal cavity.

Black found in the saliva of ten healthy individuals the Staphylococcus pyogenes aureus seven times, Staphylococcus pyogenes albus four times, and Streptococcus pyogenes three times. On the other hand, Netter found Staphylococcus aureus only seven times in one hundred and twenty-seven individuals examined. Miller also has rarely found the pus cocci in the mouths of healthy persons. Streptococcus pyogenes was not found by Vignal in his extended researches. The experiments of the writer, of Vulpian, Fränkel, Netter, Claxton, and others show that the micrococcus which in 1885 I named Micrococcus Pasteuri, and which is identical with the "diplococcus pneumoniæ" of German authors, is frequently present in the healthy human mouth—now called Micrococcus pneumoniæ crouposæ. Netter examined the saliva of one hundred and sixty-five healthy individuals and obtained it in fifteen per cent of the number examined.

Another pathogenic micrococcus which is frequently present in the mouths of healthy persons is the Micrococcus tetragenus of Koch. The following pathogenic bacteria have also been isolated and described: Bacillus crassus sputigenus (Kreibohm), Bacillus salivarius septicus (Biondi). The Streptococcus septo-pyæmicus of Biondi is described as having characters identical with those of the Streptococcus pyogenes of Rosenbach. Two other pathogenic species described by Biondi were each found in a single case only. Miller has described the following pathogenic species isolated and studied by him: Micrococcus gingivæ pyogenes, Bacterium gingivæ pyogenes, Bacillus dentalis viridans, Bacillus pulpæ pyogenes.

Rosenthal (1893) examined the secretions from the mouths of fourteen individuals and obtained twenty-eight different bacteria; of these twenty-one had been previously described. Five species believed to be new are described in detail by Rosenthal, viz.: Sarcina viridis flavescens, Micrococcus Reessii, Micrococcus ochraceus, Diplococcus Hauseri, Bacterium cerasinum.

Vignal has tested a considerable number of microörganisms, obtained by him in his cultures from the healthy human mouth, with reference to their peptonizing action upon various kinds of food, with the idea that some of them may have an important physiological function of this kind. Out of nineteen species he found ten which, after a longer or shorter time, dissolved fibrin, nine which dissolved gluten, ten which dissolved casein, and five which dissolved albumin; nine changed lactose into lactic acid, seven inverted cane sugar, seven caused the fermentation of glucose, and seven coagulated milk.

Sanarelli (1891) has shown that normal saliva has the power of destroying the vitality of a limited number of certain pathogenic bacteria, including the following species: Staphylococcus pyogenes aureus, Streptococcus pyogenes, Micrococcus tetragenus

Bacillus typhi abdominalis, Spirillum choleræ Asiaticæ. When to ten cubic centimetres of saliva, sterilized by filtration through porcelain, the above-mentioned pathogenic bacteria were added in small numbers by means of a platinum needle carried over from a pure culture, no development occurred, and at the end of twenty-four hours the bacteria introduced were incapable of growth in a suitable medium. But when this amount of filtered saliva was inoculated with a large platinum loop—an öse—a certain number of the bacteria survived, and at the end of three or four days an abundant developopment occurred. At first, however, the number of living cells was considerably diminished. In saliva to which one öse of a culture of Staphylococcus aureus was added thirteen thousand eight hundred and forty colonies developed in a plate made immediately after inoculation, while a plate made at the end of twenty-four hours contained but one hundred and thirty-two colonies, and one at the end of forty-eight hours had but eight colonies. Subsequently multiplication occurred, and a plate made on the ninth day after inoculation contained so many colonies that they could not be counted.

The diphtheria bacillus was not destroyed in filtered saliva, but did not multiply in it. On the other hand, it proved to be a very favorable medium for the development of Micrococcus pneumoniæ crouposæ.

Mucus from the surface of the *meatus urinarius* of man and woman, or from the *vagina*, will always be found to contain various bacteria ; but the bladder, the uterus, and Fallopian tubes in healthy individuals are free from microörganisms.

Winter has isolated twenty-seven different species from vaginal and cervical mucus, and reports that he found Staphylococcus pyogenes albus in one-half of the cases examined. A streptococcus was also encountered which resembled Streptococcus pyogenes, although not positively identified with it. Samschin, on the other hand, failed to obtain the pus cocci in vaginal mucus from healthy women.

Dönderlein, Von Ott, and others have carefully examined the *lochial discharge* with reference to the presence of bacteria. The first-named author found that in healthy women the lochial discharge obtained from the uterus was free from germs, but when collected from the vagina various microörganisms were obtained. In one case in which some fever existed Staphylococcus pyogenes aureus was found in the vagina, while the discharge from the uterus was free from germs. In five cases of puerperal fever Streptococcus pyogenes was obtained in the lochial discharge from the uterus. The results of Von Ott correspond with those of Dönderlein. Czerniewski, in the lochia of fifty-seven healthy women, found the Streptococcus pyogenes but once, while in the lochial discharge of fatal cases of puerperal fever it was always present.

Steffeck (1892) has examined the vaginal secretion of twenty-nine pregnant females who had not been subjected to digital examination, and found Staphylococcus pyogenes albus in nine, Staphylococcus pyogenes aureus in three, and Streptococcus pyogenes in one. These results indicate that puerperal septicæmia from self-infection may occur in exceptional cases. In seventeen of the twenty-nine cases examined none of these pyogenic micrococci were found.

Hofmeister (1894) has shown that bacteria are found not only upon the mucous membrane of the meatus urinarius in man, but that they may usually be obtained from the urethral canal at a depth of eight centimetres or more, although the number rapidly diminishes in the deeper portion of the urethra.

Walthard (1895) arrives at the conclusion that while in pregnant females bacteria are constantly found in the vagina and the lower portion of the cervical canal, they are absent from the upper part of the cervical canal, the uterus, and the tubes; and that during the puerperal condition the uterine cavity is preserved from spontaneous infection *per vias naturalis* by the plug of mucus in the cervical canal. In the vaginal secretions of one hundred pregnant women, who had not been subjected to a digital examination, streptococci were obtained twenty-seven times in cultures. These were not virulent, but, according to Walthard, these saprophytic streptococci become virulent when, owing to a diminished resisting power, they are enabled to invade the tissues as parasites.

Krönig (1894) concludes from his investigations that the vaginal secretions of pregnant women are usually so acid that Streptococcus pyogenes could not multiply in them; also that when the secretion is normal it is almost always sterile.

Döderlein (1894) insists that the failure of Krönig to obtain microorganisms in his cultures was due to the fact that suitable media were not used; also that certain bacilli are constantly found in normal, acid vaginal secretions, and that in the pathological secretions which are feebly acid, neutral, or in some cases slightly alkaline a great variety of bacteria are found, including Streptococcus pyogenes, as demonstrated by himself and other investigators. In a later paper (1894) Krönig reports his success in obtaining cultures from normal, acid vaginal secretions by using acid media and by cultivating under anaërobic conditions. He reports also that pathogenic bacteria (streptococci, staphylococci, and Bacillus pyocyaneus) introduced into the vaginæ of pregnant women lose their power of reproduction in from six to forty-eight hours (streptococci did not grow after six hours). In a still later communication (1894) Krönig reports that the bacteria present in the vaginal secretions of pregnant women are for the most part strictly anaërobic species, and that among these he found two non-pathogenic streptococci.

Menge (1894) has examined the vaginal secretions in fifty non-pregnant women who had been in bed for at least fourteen days—after laparotomy. Microscopical examination showed the presence of bacteria in all cases, but in only six cases was a development of colonies obtained—upon agar plates; in one case Streptococcus pyogenes was present. Menge concludes from his investigations that spontaneous infection during childbirth cannot occur, and that with the exception of the gonococcus the known pathogenic bacteria cannot multiply in the cervical canal.

Gawronsky (1894) has examined the secretions from the healthy urethra in sixty-two women, most of whom were under treatment for uterine disease or displacement. The material for his cultures was obtained by means of a platinum loop, introduced through a glass cylinder, at a distance of one or one and one-half centimetres from the external orifice of the urethra. In fifteen out of the sixty-two cases examined a positive result was obtained, as follows: In three cases Streptococcus pyogenes, in eight Staphylococcus pyogenes aureus, in one Staphylococcus pyogenes albus, in two Bacillus coli communis, in one Bacterium tholoideum of Gessner.

The following species have been obtained from the nasal and buccal secretions :

FROM THE NOSE.

Non-pathogenic.—Micrococcus nasalis (Hajek), Diplococcus coryzæ (Hajek), Micrococcus albus liquefaciens (Von Besser), Micrococcus cumulatus tenuis (Von Besser), Micrococcus tetragenus subflavus (Von Besser), Diplococcus fluorescens fœtidus (Klamann), Micrococcus fœtidus (Klamann), Vibrio nasalis (Weibel), Bacillus striatus flavus (Von Besser), Bacillus striatus albus (Von Besser).

Pathogenic.—Staphylococcus pyogenes aureus, Staphylococcus pyogenes albus, Streptococcus pyogenes, Bacillus of Friedländer, Bacillus of rhinoscleroma (?), Bacillus fœtidus ozænæ (Hajek), Bacillus mallei (Löffler), Bacillus smaragdinus fœtidus (Reimann).

FROM THE MOUTH.

Non-pathogenic.—Micrococcus roseus (Eisenberg), Micrococcus A, B, C, D, E of Podbielskij, Sarcina pulmonum (Hauser), Sarcina lutea, Micrococcus candicans (Flügge), Bacillus of Miller, Bacillus virescens (Frick), Vibrio rugula, Vibrio lingualis (Weibel), Pseudo-diphtheria bacillus (Von Hoffmann), Bacillus mesentericus vulgatus, Bacillus subtilis, Bacillus *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*, and *j* of Vignal, Bacillus subtilis similis, Bacillus radiciformis (Eisenberg), Bacillus luteus, Bacillus fluorescens non-liquefaciens, Bacillus ruber, Bacillus viridiflavus, Proteus Zenkeri, Bacillus G, H, I, J, K, L, M, N, and Vibrio O and P of Podbielskij, Vibrio viridans (Miller), Micrococcus nexifer (Miller), Iodococcus magnus (Miller), Ascococcus buccalis (Miller), Bacillus fuscans (Miller).

Pathogenic.—Staphylococcus pyogenes albus, Staphylococcus pyogenes aureus, Staphylococcus salivarius septicus (Biondi), Streptococcus pyogenes, Micrococcus salivarius septicus (Biondi), Micrococcus tetragenus (Gaffky), Micrococcus gingivæ pyogenes (Miller), Streptococcus septo-pyæmicus (Biondi), Streptococcus articulorum (Löffler), Micrococcus of Manfredi. Micrococcus pneumoniæ crouposæ—" Micrococcus Pasteuri " (Sternberg); Bacillus diphtheriæ (Löffler), Bacillus tuberculosis (Koch), Bacillus of Friedländer, Bacillus bronchitidæ putridæ (Lumnitzer), Bacillus septicæmiæ hæmorrhagicæ, Bacillus gingivæ pyogenes (Miller), Bacillus pulpæ pyogenes (Miller), Bacillus dentalis viridans (Miller), Bacillus crassus sputigenus (Kreibohm), Bacillus saprogenes No. 1 (Rosenbach), Bacillus pneumoniæ agilis (Schou), Bacillus pneumoniæ of Klein, Bacillus pneumosepticus (Babes).

V.

BACTERIA OF THE STOMACH AND INTESTINE.

As the secretions of the mouth contain numerous bacteria, these must constantly find their way to the stomach, but conditions are not favorable for their development when the stomach is in a healthy state and its secretions normal. Under certain circumstances, however, there may be an abundant development in the stomach of species which give rise to various fermentations, and no doubt dyspeptic symptoms are frequently due to this cause. In the present section we are, however, only concerned with the bacteria of the healthy stomach. Most of these, we think, are to be considered as only temporarily and accidentally present in this viscus as the result of the swallowing of the buccal secretions and of food and drink containing them.

The experiments of Straus and Würtz and of others show that normal gastric juice possesses decided germicidal power, which is due to the free hydrochloric acid contained in it. Hamburger (1890) found that gastric juice containing free acid is almost always free from living microörganisms, and that it quickly kills the cholera spirillum and the typhoid bacillus, but has no effect upon anthrax spores. Straus and Würtz found that the cholera spirillum is killed by two hours' exposure in gastric juice obtained from dogs, the typhoid bacillus in two to three hours, anthrax bacilli in fifteen to twenty minutes, and the tubercle bacillus in from eighteen to thirty-six hours. The experiments of Kurlow and Wagner, made with gastric juice obtained from the stomach of healthy men by means of a stomach sound, gave the following results: Anthrax bacilli without spores failed to grow after exposure to the action of human gastric juice for half an hour, but spores were not destroyed in twenty-four hours; the typhoid bacillus was killed in one hour; the cholera spirillum, the bacillus of glanders, and Bacillus pyocyanus were all destroyed at the end of half an hour; the pus cocci showed greater resisting power. Certain bacteria have a greater resisting power for acids than any of those above mentioned, and some of them may consequently pass through the healthy stomach to the intestine in a living condition, but there is good reason to believe that the spirillum of cholera or the bacillus of anthrax would not. On the other hand, the tubercle bacillus and the spores of other bacilli can, no doubt, pass through the stomach to the intestine without losing their vitality.

Of nineteen species isolated by Vignal in his cultures from the healthy human mouth, the greater number resisted the action of the gastric juice for more than an hour, and six species which did not form spores were found to retain their vitality in gastric juice for more than twenty-four hours.

In making a bacteriological analysis of the contents of the healthy stomach the more resistant microörganisms and those which form spores will naturally be found in greater or less numbers, inasmuch as some of them are likely to be present in food and water ingested.

Van Puteren (1888) obtained a variety of microörganisms in very considerable numbers from the stomachs of infants fed upon unsterilized cow's milk, but in healthy nursing infants the number was much smaller, especially when the mouth was washed out with distilled water immediately before and after nursing. In 18 per cent of the cases no microörganisms were found under these circumstances, and in 41 per cent the number fell below one thousand per cubic centimetre. Among the nursing infants examined (eighty-five) the following species were most numerous: Monilia candida, Bacillus lactis aërogenes, a non-liquefying coccus, Staphylococcus pyogenes aureus, Bacillus subtilis. In infants fed upon cow's milk (eleven) Bacillus lactis aërogenes was present in 45.4 per cent of the cases, and Staphylococcus pyogenes aureus in 27.2 per cent, non-liquefying cocci in 54.4 per cent, liquefying cocci in 72.7 per cent, Bacillus subtilis in 36.3 per cent, and Bacillus butyricus (Hueppe) in all of the cases; next to these Bacillus flavescens liquefaciens was the most abundant. The author named reaches the conclusion that no species is constant and that the presence of those found depends upon accidental circumstances.

Abelous (1889) found in his own stomach, washed out while fasting, a considerable number of species of bacteria, viz.: Sarcina ventriculi, Bacillus pyocyaneus, Bacillus lactis aërogenes, Bacillus subtilis, Bacillus mycoides, Bacillus amylobacter, Vibrio rugula, and eight other undescribed bacilli and one coccus. All of these microörganisms were able to resist the action of hydrochloric acid in the proportion of 1.7 grammes in 1,000 grammes of water. Several were found to be facultative anaërobics.

The action of the bacteria isolated by him was tested by Abelous upon various alimentary substances. The time required to effect changes, such as the digestion of fibrin, the changing of starch into glucose, etc., was found to be so long that there was no reason to suppose that any one of the microörganisms tested was concerned in ordinary stomach digestion.

In the *intestine* conditions are favorable for the development of many species of saprophytic bacteria, and the smallest quantity of excrementitious material from the bowels, spread upon a glass slide and stained with one of the aniline colors, will be found to contain a multitude of microörganisms of this class, of various forms. Among these are certain species which have their normal habitat in the intestine, and which may always be obtained in cultures from this source, while others, having been present in food or water ingested, and having escaped destruction in the acid juices of the stomach, are accidentally and temporarily present. These latter may or may not increase in the organic pabulum which abounds in the intestine, according as the conditions are favorable or otherwise. The strictly aërobic bacteria could not multiply because of the absence of oxygen, and the species encountered are for the most part anaërobics or facultative anaërobics. The Bacillus coli communis of Escherich, which is the most constant and abundant species found in the intestine of man and of certain of the lower animals, is a facultative anaërobic, which grows readily in the ordinary culture media, either in the presence of oxygen or in an atmosphere of hydrogen. But certain other bacteria of the intestine are strictly anaërobic and do not grow readily in the media commonly employed by bacteriologists.

Escherich has shown that in new-born infants the meconium is free from bacteria. At the end of twelve to eighteen hours after birth bacteria appear in the alvine discharges, and the number is already considerable at the expiration of the first twenty-four hours of independent existence. The species first found are cocci and yeast cells which no doubt come from the atmosphere, having been deposited upon the moist mucous membrane of the mouth and swallowed with the buccal secretions. When the meconium is replaced by "milk fæces" these contain in large numbers the Bacillus coli communis, heretofore spoken of as the most common species found in the intestine of adults. Another species associated with this, but not so abundant, is the Bacillus lactis aërogenes of Escherich. Other bacilli and cocci are found occasionally in smaller numbers. These bacilli do not liquefy gelatin, and, as a rule, the microörganisms found in the alvine discharges of healthy persons are non-liquefying bacteria. Escherich's researches led him to the conclusion that the Bacillus lactis aërogenes is constantly present in the small intestine of milk-fed children as the most prominent species, and that its multiplication there is favored by the presence of milk sugar, and that Bacillus coli communis finds the most favorable conditions for its growth in the large intestine.

Brieger, in 1884, isolated from fæces and carefully studied two bacilli, one of which has since been called by his name. This is a non-liquefying bacillus which is very pathogenic for guinea-pigs, and which in its morphology and characters of growth closely resembles the Bacillus coli communis of Escherich. Indeed, a number of non-liquefying bacilli, differing but slightly in their morphological and biological characters, have been obtained by various investigators from the alimentary canal of man and the lower animals, and it is still a question whether they are to be regarded as distinct species or as varieties of the "colon bacillus" of Escherich. The bacillus obtained by Emmerich from cholera cadavers in Naples belongs to this group, and, if not identical with the colon bacillus, resembles it so closely that its differentiation is extremely difficult. Brieger's bacillus forms propionic acid in solutions containing grape sugar. A second bacillus obtained by him from the same source resembles the "pneumococcus" of Friedländer; this causes the fermentation of saccharine solutions, with production of ethyl alcohol.

Bienstock (1883) isolated four species of bacilli from normal fæces, two of which are comparatively large and resemble Bacillus subtilis in their morphology and in the formation of spores. A third species is described as an extremely slender pathogenic bacillus, resembling the bacillus of mouse septicæmia. The fourth species is an actively motile bacillus which forms end spores, causing the rods to have the form of a drumstick. This is said to cause the decomposition of albumin, with production of ammonia and carbon dioxide. Later researches do not sustain Bienstock's conclusion that the bacilli described by him are the principal forms found in normal fæces.

Among the species encountered by Escherich, in addition to those mentioned above (Bacillus coli communis and Bacillus lactis aërogenes), are the following: Proteus vulgaris, found three times in meconium, and constantly in the fæces of dogs fed upon flesh; Streptococcus coli gracilis, found in meconium, but not during the period of nursing, is constantly present in the intestine when a flesh diet is employed.

The intestine of carnivorous and omnivorous animals contains a greater number of bacteria than that of the herbivora, and in the large intestine they are far more numerous than in the small intestine (De Giaxa). Sucksdorf has enumerated the colonies developing from one milligramme of fæces from individuals on mixed diet. He obtained an average of 380,000 from a series of observations in which the maximum was 2,300,000 and the minimum 25,000.

The constant presence of certain species of bacteria in the intestine of man and the lower animals has led to the supposition that they may serve a useful purpose, or perhaps even have an essential physiological rôle in connection with intestinal digestion. While this question has not been definitely settled, the experiments of Vallin, Abelous, and others have thrown some light upon it, and a recent experiment by Nuttall and Thierfelder (1895) has considerable importance as bearing upon its solution. The experiment consisted in removing a fœtus from a pregnant guinea-pig by Cæsarean section, placing it under conditions which protected it from the microorganisms present in the atmosphere, and feeding it upon sterilized milk. Great technical skill was shown in carrying out this experiment for a period of eight days, during which time the little animal was kept in a sterilized atmosphere and was fed every hour day and night. At the end of this time it had consumed over three hundred and thirty cubic centimetres of sterilized milk, and was as active and healthy as other guinea-pigs of the same age. It was now killed, and a careful bacteriological examination showed that the discharges from the bowels and the contents of the intestine were entirely sterile.

ADDITIONAL NOTES UPON BACTERIA OF THE STOMACH AND INTESTINE.

Oppler (1894) has examined material, obtained in the early morning, from the stomach of persons suffering from indigestion, and found nearly always numerous masses of sarcinæ. Five different species were obtained from this source, which were distinguished by the following characters: No. 1, colonies sulphur yellow; No. 2, colonies greenish yellow; No. 3, colonies white; No. 4, colonies white, does not liquefy gelatin; No. 5, colonies orange yellow. Nos. 1 and 3 were most frequently encountered.

Kauffmann (1895) in a carefully studied case of chronic dyspepsia obtained from the contents of the stomach in the morning before breakfast, and after a test meal, the following bacteria: Yellow sarcina, Micrococcus aurantiacus, Staphylococcus cereus albus, Bacillus subtilis, Bacillus ramosus, "a large thick bacillus," "a short bacillus resembling Bacillus coli communis." The last-mentioned bacillus was found in large numbers, and Kauffmann suggests that it may have been the cause of the fermentation in the digestive tract which caused the unpleasant symptoms in the case under investigation.

Macfayden (1887) and Gillespie (1893) have also obtained a bacillus from the stomach which appears to be identical with Bacillus coli communis. In the researches of Gillespie it was obtained from a patient with dilatation of the stomach who suffered from flatulence, etc. In all, twenty-four different microörganisms were obtained by Gillespie from the contents of the stomach of different individuals. This number includes three species of saccharomyces and a mucor. Among the conclusions reached by Gillespie are the following:

"14. Although bacteria are of no aid to peptic digestion, and a hindrance to the pancreatic ferment if in quantity in the duodenum, they still are of great use in the small intestine, where they control putrefaction. This seems paradoxical: microörganisms obstructing microörganisms but assisting digestion. It seems, however, to be true. The organisms which most easily pass the searching examination of the stomach are those which give rise by their growth to the fatty acids, as they are the most resistant to the action of acids. Their products in the small intestine are sufficient to keep the contents of that viscus acid, and they thereby prevent or control putrefaction. In the large intestine the secretion is so alkaline that the putrefactive organisms reassert themselves.

"15. Increased putrefaction in the intestinal canal may therefore be due, in some cases, either to insufficient mortality among the putrefactive organisms in the stomach, or to too great mortality among the acid-forming bacteria and yeasts.

"16. The lactic acid which appears during the first stages of digestion is due to the action of organisms.

"17. The lactic, acetic, butyric, and succinic acids found in gastroectasis are due also to organisms which luxuriate in the too stationary contents. The marsh gas, the *Brennender-gas* of the Germans, is probably due to the same cause ; in the only case of this character with which I have had the good fortune to meet no material for examination could be obtained."

The following species have been isolated from fæces and the contents of the intestine of cadavers :

Non-pathogenic.—Streptococcus coli gracilis (Escherich), Micrococcus aërogenes (Miller), Micrococcus tetragenus versatilis (Sternberg), Micrococcus ovalis (Escherich), "Yellow liquefying staphylococcus" (Escherich), "Porzellancoccus" (Escherich), Bacillus subtilis, Bacillus aërogenes (Miller), Bacterium aërogenes (Miller), Bacillus lactis erythrogenes (Hueppe), Clostridium fœtidum (Liborius), Bacillus muscoides (Liborius), Bacillus putrificus coli (Bienstock), Bacillus subtilis similis I. and II. (Bienstock), Bacillus Zopfii, Bacillus liquefaciens communis (Sternberg), Bacillus intestinus liquefaciens (Sternberg), Bacillus intestinus motilis (Sternberg), Bacillus fluorescens liquefaciens (Flügge), "Colorless fluorescent liquefying bacillus" (Escherich), "Yellow liquefying bacillus" (Escherich), Bacillus mesentericus vulgatus, Bacilli of Booker, A to T, first series; a to s, second series; Bacilli of Jeffries A to Z, and α, β.

Pathogenic.—Staphylococcus pyogenes aureus, Bacillus typhi abdominalis, Bacillus septicæmiæ hæmorrhagicæ, Bacillus of Belfanti and Pascarola, Bacillus enteritidis (Gärtner), Bacillus of Lesage, Bacillus pseudomurisepticus (Bienstock), Bacillus coli communis (Escherich), Bacillus lactis aërogenes (Escherich), Bacillus cavicida (Brieger), Bacillus of Emmerich, Bacillus coprogenes fœtidus (Schottelius), Bacillus of Utpadel, Bacillus leporis lethalis (Sternberg), Bacillus acidiformans (Sternberg), Bacillus cuniculicida Havaniensis (Sternberg), Bacillus cadaveris (Sternberg), Bacillus cavicida Havaniensis (Sternberg), Proteus vulgaris (Hauser), Bacillus tuberculosis, Spirillum choleræ Asiaticæ Spirillum of Finkler and Prior.

VI.

BACTERIA OF CADAVERS AND OF PUTREFYING MATERIAL FROM VARIOUS SOURCES.

THE putrefactive changes which occur so promptly in cadavers, when temperature conditions are favorable, result chiefly from post-mortem invasion of the tissues by bacteria contained in the alimentary canal. But it is probable that under certain circumstances microörganisms from the intestine may find their way into the circulation during the last hours of life, and that the very prompt putrefactive changes in certain infectious diseases in which the intestine is more or less involved are due to this fact. The writer has made numerous experiments in which a portion of liver or kidney removed from the cadaver at an autopsy made soon after death—one to six hours—has been enveloped in an antiseptic wrapping and kept for forty-eight hours at a temperature of 25° to 30° C. In every instance there has been an abundant development of bacteria, although as a rule none were obtained from the same material immediately after the removal of the organ from the body. This shows that a few scattered bacteria were present. The same result was obtained in cases of sudden death from accident, as from portions of liver or kidney removed from the bodies of persons dying of yellow fever, tuberculosis, and other diseases.

Numerous researches show that the blood of healthy men and animals is free from bacteria, and that saprophytic bacteria injected into a vein soon disappear from the circulation; and recent experiments show that blood serum has decided germicidal power. But in spite of this fact the experiments of Wyssokowitsch show that certain bacteria injected into the circulation may be deposited in the liver, the spleen, and the marrow of the bones, and there retain their vitality for a considerable time. The spores of Bacillus subtilis were found by the observer named to preserve their vitality in the liver or spleen of animals into which they had been injected, for a period of two or three months. In the writer's experiments the microörganisms which first developed in fragments of liver preserved in an antiseptic wrapping were certain large anaërobic bacilli, and especially my Bacillus cadaveris, together with the Bacillus coli communis of Escherich, my Bacillus hepaticus fortuitus, and other non-liquefying bacilli of the "colon group."

These bacteria did not give rise to a putrefactive odor, and the fragment of liver when cut into had a fresh appearance and a very acid reaction. Later, putrefactive changes occurred and Proteus vulgaris and other putrefactive bacteria obtained the precedence. Evidently all of these species must have been present in the liver at the time it was removed from the cadaver, although in such small numbers that they were rarely seen in smear preparations or obtained in cultures from the fresh liver tissue. The appearance of a smear preparation from the interior of a fragment preserved for forty-eight hours in an antiseptic wrapping is shown in Fig. 197.

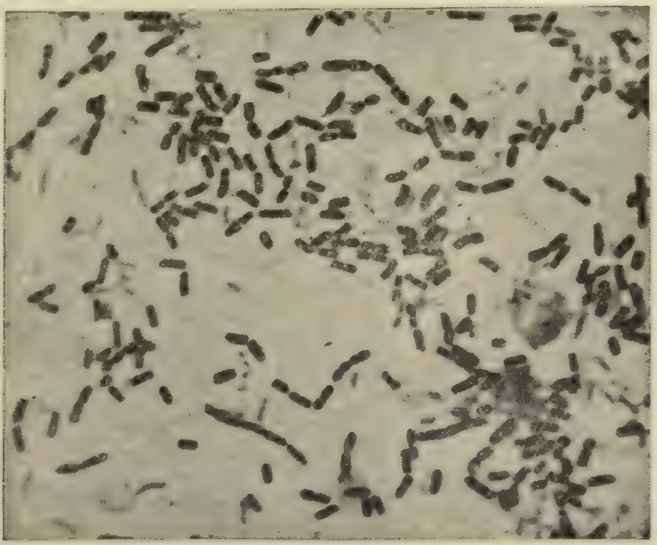


FIG. 197.—Smear preparation from liver of yellow-fever cadaver, kept forty-eight hours in an antiseptic wrapping. × 1,000. From a photomicrograph. (Sternberg.)

The horribly offensive gases which are given off from dead animals in a state of putrefaction appear to be due to certain large anaërobic bacilli which are found in such material, and which have not yet been thoroughly studied owing to the difficulty of cultivating them in artificial media; among them is a large bacillus with round ends which forms an oval spore at one extremity of the rather long rod. This the writer has described under the name of Bacillus cadaveris grandis, Fig. 198.

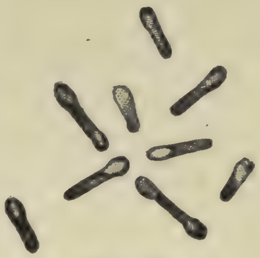


FIG. 198.

In the interior of a putrefying mass of this kind only those bacteria are found which are able to grow in the absence of oxygen, but aërobic saprophytes may multiply upon the surface of such a mass, or in organic liquids to which the air has free access. Among the most common putrefactive bacteria are the Proteus vulgaris, Proteus mirabilis, and Proteus Zenkeri of Hauser. Formerly the minute motile bacteria found in putrefying animal infusions, etc., were commonly spoken of as belonging to the species "Bacterium termo," but recent researches show that several different species were included under this name by those whose researches were made before the introduction of Koch's method for isolating and differentiating microörganisms of this class by the use of solid culture media. The different species of Proteus are all facultative anaërobics. They are more or less pathogenic, and according to Hauser produce a chemical poison which, when injected into small animals, causes death with all of the symptoms of putrid intoxication. The bacillus of mouse septicæmia, which was first obtained by Koch from a putrefying meat infusion, is also pathogenic, as are the writer's Bacillus cadaveris and various other anaërobic bacteria found in putrefying material.

Some account of the various products of putrefaction and the microörganisms concerned in their production will be found in Section IV., Part Second, of the present volume.

VII.

BACTERIA IN ARTICLES OF FOOD.

Milk always contains bacteria, unless drawn with special precautions into a sterilized flask. In the healthy udder of the cow it is sterile, but in tuberculous cows, when the milk glands are involved, tubercle bacilli may find their way into the milk in considerable numbers. As ordinarily obtained and preserved, milk is greatly exposed to bacterial contamination from various sources; desquamated cuticle from the external surface of the udder and from the hands of the milker, and floating particles from the air of the stable, fall into it at the very moment it is drawn, and it is subsequently contaminated by bacteria from the air, and from water used in washing the receptacles in which it is placed or added to it by the thrifty milkman. As it furnishes an excellent nutrient medium for many of the bacteria which are thus introduced into it, under favorable conditions of temperature it quickly undergoes changes due to the multiplication in it of one or more of these microörganisms. The acid fermentation and coagulation of the casein which so constantly occurs is completely prevented by sterilizing fresh milk in flasks provided with a close-fitting cork or cotton air filter. Numerous researches have been made with reference to the microörganisms found in milk and the various fermentations to which they give rise. Naturally a great variety of species will be found in an extended research, but all are accidentally present, and only those demand special attention which produce the various fermentations of this fluid commonly encountered, or which have special pathogenic properties.

Several different bacteria produce an acid fermentation and consequent coagulation of milk, but the usual agent in producing this fermentation is the Bacillus acidi lactici, which is identical with the "ferment lactique" of Pasteur. When a pure culture of this bacillus is introduced into sterilized milk kept at a temperature of 25° to 30° C., coagulation occurs in from fifteen to twenty-four hours. A uniform, gelatinous mass is produced which does not subsequently become dissolved (Adametz). Various other bacteria produce a similar change, including a number of common water bacteria, several species of sarcina, Staphylococcus pyogenes aureus, and other pus cocci. Usually coagulation is due to the combined action of several bacteria, among which Bacillus acidi lactici is apt to be the most prominent.

Other bacteria produce coagulation without the lactic acid fermentation. This appears to be due to the formation of a soluble ferment which acts like rennet, causing the coagulation of milk which has a neutral or slightly alkaline reaction. The coagulated casein in this case is subsequently redissolved. The bacteria which produce this change for the most part form spores, while the lactic acid ferments do not. If, therefore, milk is heated nearly to the boiling point the acid-forming bacteria will be destroyed and the spores of the other species surviving will give rise to coagulation without the production of lactic acid. Among the more common microörganisms of this group are the Bacillus butyricus (Hueppe), Bacillus mesentericus vulgatus, Löffler's "white milk-bacillus," and the bacilli described by Duclaux under the generic name of Tyrothrix.

Other fermentations are produced by certain chromogenic bacteria, and these, as a rule, are not as harmless from a sanitary point of view as those above referred to. Blue milk is produced by the presence of Bacillus cyanogenus, yellow milk by Bacillus synxanthus (Schröter) and by a species obtained by List from the fæces of a sheep and another found by Adametz in cheese. The well-known Bacillus prodigiosus produces its characteristic red pigment when present in milk, and a bluish-red color is caused by Bacterium lactis erythrogenes (Hueppe).

Viscous fermentation in milk is produced by several different bacteria, among others by a micrococcus studied by Schmidt-Mühlheim, and a short bacillus isolated by Adametz—Bacillus lactis viscosus. Milk which has undergone this change is unwholesome as food; it is recognized by the long filaments which are produced when it is touched with any object and this is slowly withdrawn.

The Caucasian milk ferment, Bacillus Caucasicus, produces a special fermentation, which has been referred to in Section IV., Part Second (page 139).

Various pathogenic bacteria have occasionally been found in milk in addition to the tubercle bacillus already referred to. Thus Adametz found Staphylococcus pyogenes aureus in two samples which had been submitted to him for examination, one of which had given rise to vomiting and diarrhœa. Wyssokowitsch cultivated from milk which had been standing some time a pathogenic bacillus, named by him Bacillus oxytocus perniciosus.

The special microörganism which produces the poisonous ptomaine called by Vaughan tyrotoxicon has not yet been isolated; nor do we know the exact cause of scarlet fever, although there is evidence that this disease has been spread by the use of contaminated milk, as have also diphtheria and typhoid fever, which diseases are due to bacilli now well known. As the cholera spirillum grows readily in milk, this disease could no doubt also be transmitted in the same way.

Sedgwick and Batchelder (1892) have examined a large number of specimens of milk obtained in Boston and vicinity, for the purpose of determining the number of bacteria present. They found, as an average of several trials, that milk obtained in a clean stable, from a well-kept cow, by milking in the usual way into a sterilized bottle, contained 530 bacteria per cubic centimetre. "When, however, the milkman used the ordinary milk pail of flaring form, seated himself with more or less disturbance of the bedding, and vigorously shook the udder over the pail during the usual process of milking," the numbers were very much higher—on an average 30,500 per cubic centimetre immediately after milking. The average of fifteen samples taken from the tables of persons living in the suburbs of Boston was 69,143 per cubic centimetre. The average of fifty-seven samples of Boston milk, obtained directly from the milk wagons and plated at once, was 2,355,500 per cubic centimetre. The average of sixteen samples from groceries in the city of Boston was 4,577,000 per cubic centimetre.

Prof. Renk found in the milk supply of Halle from 6,000,000 to 30,000,000 bacteria per cubic centimetre—a number considerably exceeding that usually found in the sewage of American cities (Sedgwick).

Cohn and Neumann (1891) have shown that the milk of healthy women frequently contains bacteria, and that Staphylococcus pyogenes albus is the species most frequently found. This has been confirmed by the researches of Palleske (1892), Ringel (1893) and others. The last-mentioned author examined the milk of 25 women recently confined, "12 of whom were healthy and 13 sick." In 3 cases only was the milk sterile; in 17 cases Staphylococcus pyogenes albus was found; in 2 cases Staphylococcus pyogenes aureus; in 1 case both albus and aureus; in 2 cases Staphylococcus pyogenes albus and Streptococcus pyogenes. The streptococci were found in a case of mild puerperal fever and in a case of phlebitis.

The researches of Hirshberger (1889), of Ernst (1895), and of others show that the milk of tuberculous cows may contain tubercle bacilli even when the udder of the animal presents no evidence of a localized tubercular infection. In 121 samples of milk examined by Ernst from 36 different cows, 19 gave a positive result; all from the milk of 12 cows in which no evidence of tuberculosis of the udder was found in a carefully made post-mortem examination. Among the bacteria which produce unwholesome changes in milk are several which cause it to become viscous or soapy. Among these we may mention Micrococcus lactis viscosus of Conn, Micrococcus Freudenreichi of Guillebeau, Bacillus mesentericus vulgatus, and Bacillus lactis saponacei of Weighmann and Zirn. A considerable number of bacilli are known which give rise to the production of butyric acid fermentation in milk and its products. Some of these are anaërobic and some aërobic. The list includes the following: Bacillus butyricus of Prazmowski, Bacillus of Liborius, Bacillus of Botkin, Bacilli of Kadrowski.

The bitter taste which milk and cheese sometimes acquire is due to the presence of special bacterial ferments; among these the best known are an aërobic, liquefying micrococcus described by Conn, a bacillus described by Weighmann, Micrococcus casei amari and Bacillus liquefaciens lactis amari of De Freudenreich (1895).

In fresh *butter* of good quality comparatively few microörganisms are found, but the researches of Conn show that the characteristic and agreeable flavor of fresh butter is due to, or at least may be imitated by, a bacillus which is concerned in the ripening of cream under normal conditions. Cultures of this bacillus (Bacillus 41 of Conn) have already been used in a practical way by butter makers to improve the flavor of their product.

Kreuger (1890) obtained from "cheesy butter," having a disagreeable odor, various bacteria. Among these the most numerous were an oval micrococcus (Micrococcus acidi lactici, Kreuger), a slender bacillus resembling Bacillus fluorescens, and Bacillus acidi lactici of Hueppe.

Klecki (1894) has isolated from rancid butter several bacteria not previously described, one or more of which are no doubt concerned in the production of the rancid taste and odor. These are described under the following names: Bacillus butyri, Diplococcus butyri, a bacillus resembling Iodococcus vaginatus of Miller, Tetracoccus butyri, Bacillus butyri No. 2.

Duclaux (1887) has isolated from different kinds of *cheese* no less than eleven different species of bacteria, which he believes are concerned in the "ripening process." Seven of these are aërobic and four anaërobic species. Adametz (1889) has also isolated and studied a number of species to which he attributes the ripening of cheese.

More recently Henrici (1895) has studied the bacterial flora of cheese, and Marchal (1895) has shown that the ripening of certain kinds of cheese (fromages mous) is probably due to Oidium lactis.

Meats, even when salted and smoked, may contain living pathogenic bacteria which were present prior to the death of the animal, and, when not properly preserved, are of course liable to be invaded by putrefactive bacteria.

The researches of Foster (1889) show that the typhoid bacillus, the pus cocci, the tubercle bacillus, and the bacillus of swine plague resist the action of a saturated solution of salt for weeks and even for months; and the same observer found that the ordinary processes of salting and smoking did not destroy the tubercle bacillus in the flesh of a cow which had succumbed to tuberculosis. Beu has made cultures from a large number of specimens of fresh, salted, and smoked meats and fish, with the general result that the fresh and salted meats were found to contain a limited number of bacteria of various species, and that smoking for several days did not insure the destruction of these microörganisms. In specimens of sausage six days' smoking did not destroy a liquefying bacillus which was present, but at the end of six weeks' exposure to smoke this bacillus no longer grew, while a non-liquefying bacillus present in the same specimen had not been destroyed. Fourteen days' smoking sufficed to destroy all the microörganisms in a specimen of bacon, but this was not sufficient for the interior portions of a ham. Among the bacteria obtained by Beu from smoked meats he mentions the following: Staphylococcus cereus albus, Proteus vulgaris, Staphylococcus pyogenes aureus, Bacillus liquefaciens viridis, etc. The number of colonies which developed from a fragment, the size of a mustard seed to that of a flaxseed, taken from the interior of the meats examined, was usually small; and the presence of a few scattered bacteria of these common species has no significance from a sanitary point of view, except as showing that pathogenic bacteria may survive in infected meats after they have been exposed to the usual processes of salting and smoking.

Petri, in experiments upon the bacillus of swine plague (Schweinerothlauf), arrived at the following results:

The flesh of swine which died of this disease preserved its infectious properties after having been preserved in brine for several months, and the same flesh salted or pickled for a month and then smoked for fourteen days contained the rothlauf bacillus in a living and unattenuated condition. At the end of three months virulent rothlauf bacilli were still obtained from a smoked ham, but they were no longer found at the end of six months.

Schrank (1888) has made cultures from both the albumin and the yolk of *fresh eggs*, and finds that they are free from bacteria. He thinks that, as a rule, putrefactive bacteria obtain access to the interior through injured places in the shell, although exceptionally the egg may be infected with them in the oviduct of the fowl. The usual bacteria concerned in the putrefactive changes in eggs are, according to the author mentioned, a variety of Proteus vulgaris and Bacillus fluorescens putidus.

Zörkendorfer (1893) has cultivated from rotten eggs sixteen different bacilli, all of which are described in detail and none of which were found to correspond with previously described species as given in Eisenberg's Bacteriological Diagnosis.

Peters (1889) has studied the flora of the "sauerteig" used in Germany as yeast for leavening bread. In addition to the numerous cells of three species of Saccharomyces, he finds that bacilli are present in great numbers, as shown by direct microscopical examination and culture experiments. He describes five species, designated Bacillus A, B, C, D, and E, which are commonly present, and to which the acid fermentation of the dough is ascribed.

In Graham bread which had undergone changes making it unfit to eat, Kratschmer and Niemilowicz have found the Bacillus mesentericus vulgatus, which appears to have been the cause of the fermentation, which was produced in bread having a slightly alkaline reaction by inoculating it with a pure culture of this bacillus. The infected bread has a brownish color, a peculiar odor, and becomes sticky and viscid.

Uffelmann (1890) has also studied the bacteria in spoiled rye bread, and obtained, in addition to common mould fungi, Bacillus mesentericus vulgatus and Bacillus liodermus.

Waldo (1894) has shown that baking does not sterilize bread. This was to have been expected in the case of the spores of bacilli, but it is somewhat surprising to find that two species of Sarcina and two micrococci survived the baking process. In all Waldo obtained thirteen species of bacteria from the interior of sixty-two loaves examined. Bacillus subtilis and allied spore-forming bacilli were most frequently found, and the statement is made that a loaf "from a low-class, dirty bakery will almost invariably contain more living bacteria (or their spores) than one from a good, clean bakery."

Lehmann (1894) under the name Bacillus levans has described a microörganism which closely resembles Bacillus coli communis. This was obtained from sour dough, and was believed to be the cause of the acid fermentation which so often interferes with success in obtaining sweet and wholesome bread. When a culture of this bacillus was added to flour and water, without the addition of yeast, an active fermentation occurred and the dough became acid.

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